Severe Trauma and Sepsis

Organ Damage and Tissue Repair

Xiaobing Fu
Liangming Liu
Editors

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Preface

The prevention, early management, tissue repair, regeneration and rehabilitation of severe trauma are a systematic project with great clinical and social significance. In recent years, great success has been achieved not only in China but also in the world. In the whole management process, early life saving and later rehabilitation management play a key role in effective, timely, and high-quality treatment of severe trauma.

In 2017, we have published a book titled *Advanced Trauma and Surgery*. That book was a summary of new advances in trauma and surgery, especially in early medical rescue, wound care, traumatic or burn shock, pathogenesis of sepsis, and tissue repair and regenerative medicine. However, many aspects of trauma were not included in that book, such as severe complications, wound care, and latest advances of stem cells in wound management. Thus, it is our desire that another book be published to make up for this deficiency; hence this new book titled *Severe Trauma and Sepsis: Organ Damage and Tissue Repair* was edited and published. In this book, there are 20 chapters and some new advances such as damage control in wound management and stem cell application in trauma treatment are included. The main theme of this book is to promote the understanding of these new advances and potential applications in basic research and clinical use. The authors contributed to this book are scientists or doctors from fields such as early trauma rescue, vascular surgery, cardiology, neurology, orthopedics, burns, plastic surgery, rehabilitation medicine, stem cells, and biomaterials. All of them are very experienced in their professional fields, and most of the content are their own work.

We would like to thank all the authors for their contribution to this new book. Also, our special thanks go to all those who have provided support for the successful publication of this book.

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Now, he is the Chairman of the Asian Wound Care Association (AWCA), the President of the Chinese Tissue Repair Society (CTRS), President of the Chinese Tissue Repair and Regeneration Society (CTRRS), and the President of Chinese Society for Biomaterials.Professor Fu was awarded the International Lifetime Achievement Award in Wound Repair and Regeneration in 2008. He was selected as the Academician (Chinese Academy of Engineering, Division of Medicine and Health) in 2009 and Foreign Member of French Academy of Medicine in 2018.

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desensitization mechanism of vascular hyporeactivity for critical illness such as severe trauma and shock and the prevention and treatment measures; raised the new concept of permissive hypotensive resuscitation for uncontrolled hemorrhagic shock; and developed a series of emergent care devices for war wound and trauma. He has published over 350 papers in journals such as *Ann Surg*, *Cardiovasc Res*, *Crit Care Med*, *Crit Care*, *Anesthesiology,* and *Shock* and has obtained 12 national and provisional science and technology progress awards.

Damage Control in Abdominal Compartment Syndrome

1

Cheng Zhao and Jianan Ren

Abstract

Abdominal compartment syndrome (ACS) is the endpoint of increased intra-abdominal pressure (IAP) which is the result of massive interstitial swelling in the abdomen or rapid development of a space-filling lesion within the abdomen. The intra-abdominal hypertension (IAH) leads to decreased abdomen perfusion pressure (APP) resulting in abdominal viscera dysfunction contributing to multi-organ dysfunction (MOD) and ischemia which lead to high mortality. Measurement has been taken to monitor the IAP for the contradiction between resuscitation and the massive interstitial swelling which lead to IAH. Besides the monitor measurements, damage control was introduced to save the severely injured patients who are on the edge of physiological limit. Damage control resuscitation and damage control surgery were conducted to maintain the balance among physiological limit, resuscitation, and controllable IAP. There is minimal original article about the pathophysiology of ACS. Most results were from clinical trial. Many early studies of IAH and ACS used discordant definitions or cutoff pressure values. In this review, nomenclature will follow the terminology established by the World Society of the Abdominal Compartment Syndrome (WSACS) which has recently been standardized and accepted widely. This chapter reviewed the history and the pathophysiology of ACS and the application of damage control.

Keywords

Abdominal compartment syndrome · Damage control surgery · Intra-abdominal hypertension · Multi-organ dysfunction · Open abdomen

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Abbreviations

ACS	Abdominal compartment syndrome
APP	Abdominal perfusion pressure
ATLS	Advanced trauma life support
IAH	Intra-abdominal hypertension
IAP	Intra-abdominal pressure
ICU	Intensive care unit
MAP	Mean arterial pressure
MTP	Massive transfusion protocol
OA	Open abdomen
PCD	Percutaneous catheter drainage
PEEP	Positive end-expiratory pressure
PPV	Pulse pressure variation
TAC	Temporary abdominal closure
tPA	Tissue plasminogen activator
WSACS	World Society of the Abdominal Compartment Syndrome

1.1 Introduction

Irrespective of etiology, the interstitial swelling and rapid space-filling would lead to increased intraperitoneal pressure (IAP). ACS was introduced to emphasize the role of abnormal IAP in the pathology and high mortality. The standardized criteria were proposed within 15–20 years by World Society of the Abdominal Compartment Syndrome (WSACS) [1]. Defined terms and recommended treatments were presented. In light of the developed recognition of ACS, damaged control resuscitation and damage control surgery were taken into consideration for the management of critically ill surgical and/or medical patients. Damage control procedures (e.g., percutaneous catheter, catheter) was taken to monitor the IAP. In this article, new progress in ACS and damage control would be reviewed.

1.2 Abdominal Compartment Syndrome

Increased attention on IAP has led to an exponential growth in research relating to intra-abdominal hypertension [2–5]. Changes in the management of critically ill patients promote the changes in ACS with increased notice of damage control. The risks of excessive resuscitation and initial surgery were evaluated. The role of IAP, as the etiology, has been duly noted though the potential pathology is not clear. Damage control procedure was taken to monitor IAP and avoid MODs. This part will introduce the ACS and its relation with MODs.

1.2.1 Pathophysiology of ACS

In the nineteenth century, Marey et al. proposed that intrathoracic pressure could intact with IAP, and Bert et al. tested the hypothesis through animal study. The result showed the importance of diaphragmatic descent in the increase of IAP. In 1911, Wendt found that the higher IAP was, the less the urine volume would be. Heinricius et al. found that if the IAP >27~46 cm H_2O, there was more potential possibility in developing respiratory failure. After that, there were more articles about the internal connecting link between IAP and organ failures. The definition of IAH was not proposed until 1984 [5–7]. There were many definitions and criterions about ACS and IAH. However, the definitions were not standardized and unified until recent years. Among the different definitions and criterions, the definitions and criterions formulated by WSACS were widely accepted. WSACS is a highly focused specialist society. It has been assisting healthcare workers to better understand IAH and ACS; the WSACS's efforts have certainly contributed to many advances that have been made in the past.

The primary cause should be owing to the abdominal viscera swelling and/or the rapid filling of abdominal cavity by bowel edema and accumulated blood and clot. Besides these, medical interventions such as massive fluid resuscitation and forced closure of a noncompliant abdominal wall are also common hazards. And that's the reason why researches emphasized the importance of damage control resuscitation and damage control surgery (open abdomen).

The clinical manifestation changes when different organs involved. Different organs were introduced, respectively. However, whatever the manifestation changes, when IAH or elevated IAP is associated with organ dysfunction, there is ACS.

1.2.1.1 Pulmonary Dysfunction

IAP has direct impact on pulmonary function through elevating hemidiaphragm. When IAP is over 15 mmHg, the hemidiaphragm could be elevated which results in increased intrathoracic pressure, hypoventilation, and respiratory failure. Progressive reduction in total lung capacity, functional residual capacity, and residual volume could be detected as the result of the impaired pulmonary compliance. Alveolar oxygen tension was reduced, and intrathoracic pressure was increased. All these contributed to the pulmonary vascular resistance which led to hypoxia, hypercapnia, and increasing ventilatory pressure. Then pulmonary dysfunction appears. In this case, surveillance for IAH and ACS was very important. Once the ACS or IAH was detected, interventions to decompress the abdominal cavity should be performed, and the pulmonary dysfunction could be reversible [8–10].

1.2.1.2 Cardiac Dysfunction

IAP caused cardiac dysfunction through increased intrathoracic pressure. IAP could be transmitted to the intrathoracic pressure by upward bulging of the hemidiaphragms. Increased intrathoracic pressure contributed to the cardiac compression, reduced

inferior and superior vena cava flow, and reduced end-diastolic volume. When the IAP kept increasing, the patient's condition can be worsened. If the IAP is over 20 mmHg, the systemic vascular resistance would be too high for keeping a normal cardiac output and blood pressure. All these disorders could lead to the reduction in stoke volume. It can be compensated by the increase of heart rate and vascular contractility. However, the increased vascular contractility raised the systemic vascular resistance in return. The Starling curve shifted down and to the right, and cardiac output progressively falls with increasing IAP. The patient's condition could be worse if the patients combine with concomitant hypovolemia. All these could lead to the shifted Starling curve and gradually decreased cardiac output [8]. To prevent such situation, store volume, central venous pressure, and pulmonary artery wedge pressure should be monitored. In recent years, minimized invasive procedures, ultrasonic cardiac output monitor, e.g., have been developed to monitor the cardiac output. The damage control idea developed with the minimized invasive ideas, too. Damage control was set for severely injured patients who cannot receive surgery at once. The core idea was to limit the damage and restore physiological status. The development of minimized invasive was a supply to damage control concrete measure [9, 10].

1.2.1.3 Renal Dysfunction

The IAH-related renal dysfunction was prerenal and renal, and it's multifactorial. The damage of renal is progressing. When the IAH is in the range of 15–20 mmHg, the urine output will decline to less than 400 ml/day. When the IAH is above 30 mmHg, it will be anuria [10]. The prerenal factor could be owing to cardiac dysfunction. Altered cardiac function and reduced cardiac output changed renal plasma flow and glomerular filtration rate which are fundamental for renal function. The renal factor could be owing to the renal parenchymal compression. The alterations in renal arterioles and veins induced the impairment of renal function which leads to the secretion of hormone, and the hormone, aldosterone, e.g., increased vascular resistance in return. All these factors highlighted the importance of monitoring IAP and abdominal perfusion pressure (APP). Filtration gradient (FG) should be monitored as MAP-2 × IAP. They reflected the real plasma flow and determine the urine volume. To monitor IAP and APP is to save organs [11–15].

1.2.1.4 Gut and Liver Dysfunction

Liver and gut were influenced by IAH directly, especially the gut. When IAP is above 10 mmHg, the high pressure compressed the vein and artery including arteriae mesenterica superior, arteriae mesenterica inferior, and portal vein system. Mesenteric blood flow is sensitive to the graded elevation in IAP. The blood flow is approximately 70% of baseline at 20 mmHg. Perfusion is changed and intestine mucosa is very sensitive to the change. Lack of perfusion and reperfusion injury after resuscitation would aggravate the injury resulting in physiologic gut mucosal barrier dysfunction, loss of tight junction, and bacterial translocation. And the gut has been hypothesized to be the motor of multiple organ dysfunction syndrome for a long time. If the gut dysfunction is not spotted early, sepsis could be expected. If the gut disorder is not corrected early, the patients may not have good prognosis, and chronic critical duration of disease could be expected [15–19].

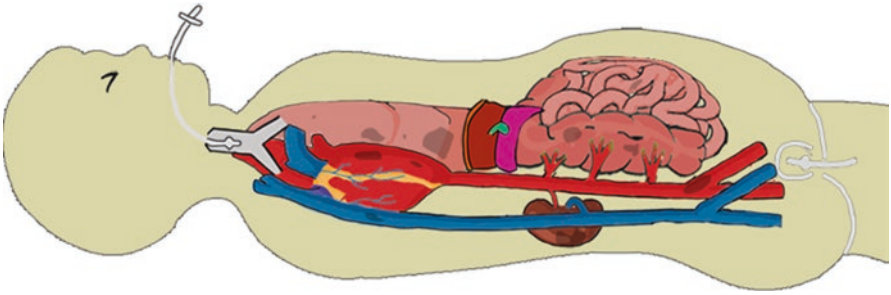

Fig. 1.1 IAP 12-15 mmHg, increasing physiologic compromise (Abviser.com). Very subtle clinical signs could be found. During this phase, sedation, pain control, nasogastric tube, rectal tube, enemas, and bowel prokinetic should be applied. Fluid resuscitation and mechanical ventilation should be carefully assessed

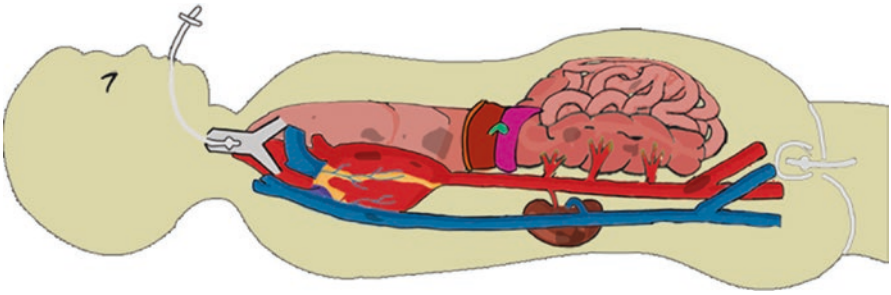

Fig. 1.2 IAP 16–20 mmHg, occult organ ischemia (Abviser.com). The patients showed acidosis, elevated CVP and wedge pressure, decreased cardiac pressure, decreased urine output, hypoxemia, hypercarbia, and atelectasis. Abdominal distension might be visible. Enteral nutrition should be limited. Excessive fluid must be removed. Measures include diuretics, hemofiltration, or dialysis. Paracentesis catheter could be used to drain the free fluid under CT or ultrasound

The liver encounters similar situation if the IAP is above 20 mmHg. The splanchnic vascular resistance is the major determinant. The IAH changed systemic hemodynamics and lead to decreased APP. To save organs and avoid ACS, risk factors must be evaluated, and IAP should be measured before the IAH developed.

Before evaluating multi-organ dysfunction, the occult ischemia may show little manifestation. The occurrence of ACS may be rapid and occult which highlights the importance of treating patients according to accurately monitored IAP [20] (Figs. 1.1, 1.2, and 1.3).

1.2.2 Measurement of IAP

IAP changes according to the abdominal content and compliance. The normal range of IAP is 0–5 mmHg. Physiologic factor such as cough and obesity could lead to the increase of IAP. In terms of obesity, the IAP could increase to 14 mmHg. The

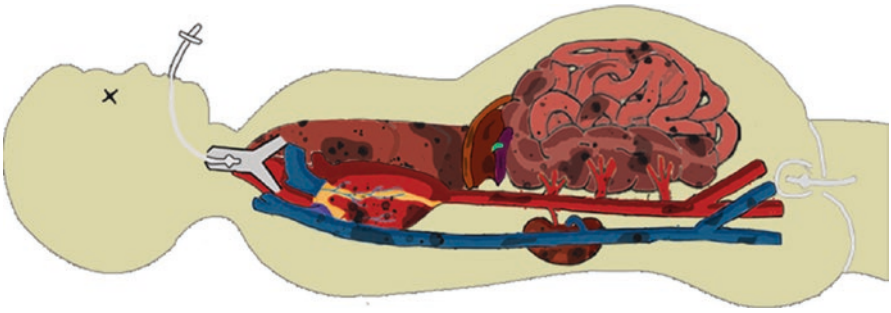

Fig. 1.3 IAP>20 mmHg, onset of multiple organ dysfunction syndrome (Abviser.com). Refractory acidosis and multi-organ failure could be observed. Neuromuscular blockade and infusion should be used to evacuate the compression. Any measure that helps relieve the IAP could be taken including stopping enteral nutrition, colonoscopy to decompress colon, and abbreviated surgery

change of IAP in the obesity and the pregnant is not clear. For common sense and research purpose, it is widely accepted that when the IAP is above 12 mmHg, it should be defined as IAH. The manifestation could be severe abdominal distention, pulmonary dysfunction, refractory hypercapnia, renal failure, etc [21, 22]. If the IAH is not corrected in time, the mortality could be rather high. To monitor is to save. Before 2006, the definition of IAP, IAH, and ACS is not unified which made the clinical research study lack statistical meaning. To solve the problem, WSACS unified the definitions in 2006 and revised the definitions in 2013. And the IAP was defined as steady-state pressure concealed within the abdominal cavity. It should be expressed in mmHg and measured at end-expiration in the supine position after ensuring that abdominal muscle contractions are absent and with the transducer zeroed at the level of the midaxillary line.

The risk evaluation is very important. When the patient was admitted to the hospital, the risk factors should be evaluated including the decreased abdominal compliance, increased digestive tract content, increased abdominal content, capillary leakage, and fluid resuscitation. If the patent has more than two risk factors, the measure of IAP was immediate and necessary. The measurements include direct and indirect measurements. In terms of direct measurements, this kind of approach will do more damage to patients. It is widely accepted that indirect measurements are acceptable. It could be assessed with placement of an indwelling catheter with a fiber-optic strain gauge pressure transducer, a nasogastric tube, or an inferior vena cava catheter (Fig. 1.4). And it also could be detected directly with intraperitoneal catheter through pressure sensors. However, the most important thing is not the way of measurement but the time to measure [9–15].

If the risk factors are more than two, the measure should be conducted. If the IAH is >12 mmHg, initial treatment should be conducted to reduce IAP and optimize organ perfusion. During this procedure, excessive fluid resuscitation should

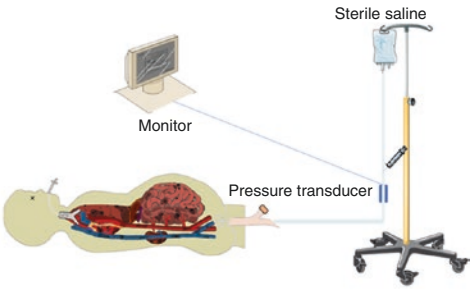

Fig. 1.4 Intra-abdominal pressure measurement using the pressure transducer technique

be avoided. And it should be monitored every 4 h. If the IAP is >20 mmHg with associated organ failure, ACS could be confirmed, and etiology should be corrected. When the IAP is <12 mmHg continuously, IAH has been solved. Frequency of IAP measurements should be decreased and observe patient for deterioration.

1.3 Damage Control

The definition of damage control originated from the Navy to save ships which are damaged during the launching out. Allowing the exist of impairment with limited repair, the ship could be salvaged under the dilemma. The concept of damage control was further developed and introduced into medical domain when patients were severely wounded and on the edge of physiological limit [23].

Damage control was then established as a potential procedure to save critical-injured patients with limited intervention. The inner idea of damage control is that the prognosis is closely related to patients' physiological limit. Even perfect surgeries could be great hit to patients. In critical patients, the "lethal triad" of acidosis, coagulopathy, and hypothermia is presented as a vicious cycle. When the lethal triad appears, the physiological limit is marked. Damage control was established to interrupt the vicious cycle, improve the condition, and prepare for the final, crucial surgery. In 1993, Rotondo concluded the researches and found that although the mortality of damage control can still be high, up to 52%, the critically ill patients without damage control almost all died. It was believed that damage control could avoid physiological exhaustion and the hit from traditional surgery. ACS usually combines with severe infection, massive hemorrhage, or severe malnourishment which means the ACS patients have lower physiological limit. All these factors underline the importance of damage control. Damage control could avoid additional physiological expenditure and bleeding [24].

The management of IAH/ACS could be divided into two parts including noninvasive measures and invasive measures. Damage control is not only the idea about treating severe patients. It should be the idea that treating specific patient with particular measures without further iatrogenic injury.

1.3.1 Initial Treatments

When the IAH appears, noninvasive measures could be used to relieve the pressure without damage to body. The reasons of increased IAP could be increased intraluminal contents, increased intra-abdominal space-occupying lesions, decreased abdominal wall compliance, and excessive fluid resuscitation. There are several approaches to deal with the potential reasons. To evacuate the intraluminal contents, inserted nasogastric and/or rectal tube prokinetic agents could be used to relieve the enteric pressure (Fig. 1.5). Besides prokinetic agents, enteral nutrition should be balanced, and enemas could be necessary. If regular drugs are not useful, colonoscopic decompression and discontinuation of enteral nutrition should be considered. To evacuate the abdominal compliance, sedation and analgesia are applied. After sedation and analgesia are applied, patients with IAH could remove the constrictive dressings and abdominal eschars. Neuromuscular blockade is useful for decreasing abdominal compliance. Body position like reverse Trendelenburg is also helpful. Fluid resuscitation plays an important role in ACS treatment. However, excessive fluid resuscitation should be avoided. At day 3, zero to negative fluid balance should be achieved according to hemodynamics. Hypertonic fluids and colloids work well with diuretics. If the patients manifest renal failure, hemodialysis or ultrafiltration is the last resort [24–27].

If IAP is above 20 mmHg and new organ failure/dysfunction is present, patients' IAH/ACS don't response to the treatment; invasive measures should be taken into consideration.

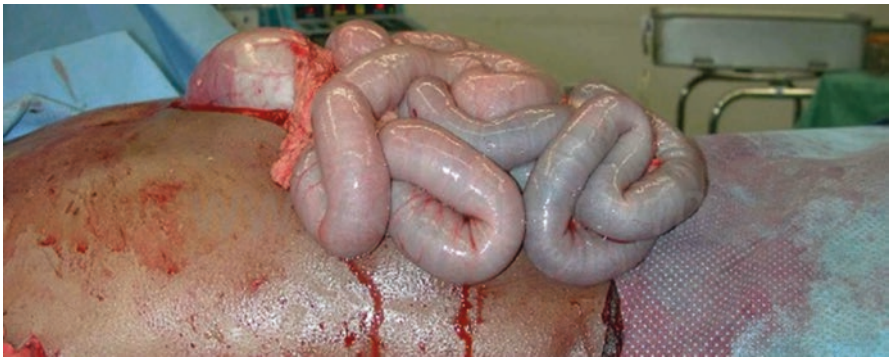

Fig. 1.5 Excessive fluid and enteral content will lead to the swelling of the intestine. All these factors are fatal in IAH

1.3.2 Abbreviated Surgery

Abbreviated surgery includes open abdomen (OA) and percutaneous catheter drainage (PCD) involving abdominal paracentesis and peritoneal lavage with trocar.

Percutaneous catheter drainage was recommended in guideline to remove fluid, and it can alleviate the need for decompressive laparotomy. When there is intra-abdominal sepsis, open abdomen should not be routinely utilized. If the patient manifest intra-abdominal sepsis, double cannula douche and negative pressure drainage are superior to the percutaneous catheter drainage. Open abdomen was first introduced as temporary abdominal closure for those whose abdomen could be closed at once in the 1940s.With development of temporary closure, open abdomen was established as a usual way to control ACS, hemorrhagic shock, mesenteric ischemia, and intra-abdominal sepsis [26, 27].

Critically ill patients usually have lethal triad including hypothermia, acidosis, and coagulopathy. Thus, the primary goal of damage control was to control hemorrhage and contamination. The reconstruction of abdominal anatomic structure could be delayed until vital signs were corrected and perfusion was restored. Only if the physiology was restored, the definitive repair could be operated. The damage control strategy stabilizes the patients' vital signs and avoids multi-hits to patients. After correcting the lethal triad, especially the coagulopathy, the mortality could be reduced. The primary acute coagulopathy of trauma is aggregated by hypothermia and acidosis which made even adequate perfusion with FFP meaningless. The low temperature and pH made the coagulation factor dysfunction and thrombin generation impaired. What's more, the critically ill patients in hemorrhagic shock are resuscitated with crystal liquids and colloidal liquids. As the result, platelet-poor plasma exacerbates coagulopathy and high mortality. Fresh frozen plasma (FFP), packed red blood cells (PRBCs), and platelets in a 1:1:1 ratio could limit further coagulopathy from the lethal triad. All these emphasize the importance of early diagnosis of lethal triad and the role of damage control.

The study of physiological parameters in open abdomen has been continued for decades. And temporary abdominal closure has been proposed after the parameters are normalized. An ideal temporary abdominal closure should decrease the IAP, promote adequate peritoneal drainage, protect the abdominal contents, especially the guts, and accelerate the close procedure. TAC could be divided into three kinds including skin closure, fascial closure, and negative pressure assisted abdominal closure. The negative pressure assisted abdominal closure has proved its superiority to skin closure and fascial closure with lower incidence of enteric fistulae.

Bowls, light canvas, and plastic were used for temporary abdominal closure. These materials are rough, airtight, and undegradable which led to secondary damage to abdomen and secondary infection. They can only protect the intestine from mechanical damage, but they cannot accelerate the healing of the wound. At last, the wound cannot be closed due to adhesion, high tension, severe intra-abdominal infection, and muscle contracture. There are more people casting their lights on early abdomen closure with biological materials these years,

hydrogel and decellularized tissue, e.g. Composite materials were produced to accelerate the healing process. Growing factors like rbFGF and rhGH were integrated into the polypropylene mesh or hydrogel and showed great ability in boosting healing [28]. In situ hydrogels with enhanced tissue regeneration ability were introduced with marked neovascularization and thicker thickness. The biomaterials have enhanced ability of promoting healing, antimicrobial, and degradable. Flexible and biocompatible ability could be expected owing to the high molecular polymerized structure which means better protection of the wound. Besides the hydrogel, electrospun scaffold has been used for the porous structure which mimics the extracellular matrix. Poly(ester urethane) urea and polycaprolactone could be used to produce the nanofibers. The electrospun showed great biocompatibility and cell adhesion. It has been proved as good scaffold for cell proliferation and function in abdominal tissue regeneration, corneal cells planting, and cardiac muscle cell growing directional array, although many biomaterials have been proposed in tissue engineering. Most experiments were conducted on rat model. The adverse events, such as intestinal fistula and abdominal tissues adhesion, couldn't be ignored if the materials are going to be applied on human [29–35].

1.3.3 Damage Control Resuscitation

The severely damaged ACS patients combine with hemorrhage and lethal triad. Before damage control resuscitation, abbreviated surgery should be done to decompress the IAH or ACS, correct the pathogeny, and repair the anatomical defect with limited damage. Besides open abdomen, damage control strategy also encompasses any approach that was used to minimize the iatrogenic injury and stabilize the physiological state. After the vital signs are corrected, damage control resuscitation should be carried to eliminate the lethal triad which is to stabilize the physiological state. In 1993, the landmark article written by Rotondo et al. has proved that this approach showed sevenfold survival rate in ACS patients.

Two key components of damage control resuscitation are permissive hypotension and hemostatic resuscitation. Recent researches advocated limited fluid resuscitation and used blood products earlier to correct coagulopathy. The advanced trauma life support (ATLS) has proposed that the initial resuscitation could be decreased from 2 L to 1 L and blood or blood products should be used regardless of specific ratio. It was designed for the hemorrhagic patients whose hemorrhage is not controllable and the patients who didn't respond to the initial fluid resuscitation. Large volume of isotonic fluid and packed red blood cells (PRBCs) should be avoided, for they can aggregate the coagulopathy by causing hemodilution and hypothermia [36–38].

1.3.3.1 Permissive Hypotension

At first, people started to realize the importance of resuscitation with massive fluid resuscitation. Crystalloid resuscitation and colloid resuscitation were widely used

three times of the loss blood. The hemorrhagic patients received large volume fluid resuscitation to maintain perfusion and vital signs.

With the deepening cognition of shock, people start to realize that intravenous fluid resuscitation could worsen uncontrolled hemorrhage by disrupting the colt formation, hemodilution, hypothermia, etc. [39] Besides the physiology disorders, the cellular swelling caused by massive fluid resuscitation could lead to inflammatory cascades which would aggregate abdominal compartment syndrome and multi-organ failure. Permissive hypotension was designed to minimize the intravenous fluid and maintain the subnormal blood pressure and end-organ perfusion. The ninth ATLS curriculum recommended that the initial fluid resuscitation should be decreased from 2 L to 1 L. During the resuscitation, doctors should pay more attention to hemodynamics. In terms of hemorrhage, the aggressive fluid resuscitation may lead to secondary bleeding. When it comes to ACS, it both can increase bleeding risk and aggregate the compression. The damage control resuscitation was recognized as delayed fluid resuscitation at first. When damage control resuscitation was applied, organ state should be concerned including urine output, systolic pressure, cardiac output, and so on. It combines with increased incidence of shock and risk of multi-organ failure. Under this condition, if there is head injury, the restricted fluid resuscitation cannot be used for cerebral perfusion may be lower then [40].

1.3.3.2 Hemostatic Resuscitation

The core part of hemostatic resuscitation is to recognize the acute coagulation. In ACS, bleeding is one of the most regular complications. Hemostatic resuscitation was designed to empirically restore clotting factors and stop bleeding with fresh frozen plasma, platelets, and PRBCs. Massive transfusion protocol (MTP) was set up for avoiding hypothermia, metabolic acidosis, dilutional coagulopathy, etc. It's defined as transfusion of ten or more units of red blood cells in less than 24 h and can be lifesaving for the severely injured. In these severely injured patients, coagulation is common and difficult to correct. Early and intensive resuscitation therapy with blood products is associated with better outcomes. Plasma coagulation factor activity should be kept above 40%, and platelet counts should be in a range of 50–100 × 10^9. In ACS, the patients with thrombocytopenia should be recognized when admitted into hospital for highly risk of bleeding and infection. With the deeper recognition of MTP, the ratio of FFP, RBC, and PLT was changed. Improved survival in traumatic patients with higher FFP was found. Recent change opinions about MTP emphasized that there is not a single solution that fits all [40–42].

1.4 Conclusion

ACS is a highly morbid disease process with elevating IAP. The prognosis is closely related with physiological limit. Damage control was applied to avoid reaching physiological limit and further damage. The procedures include open

abdomen, minimized invasive options, and damage control resuscitation. Less fluid resuscitation with crystalloid and earlier transfusion of blood products are recommended. The ratio of blood products is changed, too. Permissive hypotension is supported in consideration of coagulopathy. Additional studies on management of damage control measurements on IAH/ACS with different hemodynamics are needed.

References

1. Kirkpatrick AW, Roberts DJ, De Waele J, Jaeschke R, Malbrain ML, De Keulenaer B, Duchesne J, Bjorck M, Leppaniemi A, Ejike JC, et al. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the world Society of the Abdominal Compartment Syndrome. Intensive Care Med. 2013;39(7):1190–206.
2. Bailey J, Shapiro MJ. Abdominal compartment syndrome. Crit Care. 2000;4(1):23–9.
3. Van Hee R. Historical highlights in concept and treatment of abdominal compartment syndrome. Acta Clin Belg. 2007;62(Suppl 1):9–15.
4. De Santis L, Frigo F, Bruttocao A, Terranova O. Pathophysiology of giant incisional hernias with loss of abdominal wall substance. Acta Bio-Medica: Atenei Parmensis. 2003;74(Suppl 2):34–7.
5. Bradley SE, Bradley GP. The effect of increased intra-abdominal pressure on renal function in man. J Clin Invest. 1947;26(5):1010–22.
6. Bradley SE, Mudge GH, Blake WD, Alphonse P. The effect of increased intra-abdominal pressure on the renal excretion of water and electrolytes in normal human subjects and in patients with diabetes insipidus. Acta Clin Belg. 1955;10(3):209–23.
7. Zhang AK. The potential participation of abdominal pressure in preeclampsia. Med Hypotheses. 2015;84(6):583–5.
8. Ridings PC, Bloomfield GL, Blocher CR, Sugerman HJ. Cardiopulmonary effects of raised intra-abdominal pressure before and after intravascular volume expansion. J Trauma. 1995;39(6):1071–5.
9. Kron IL, Harman PK, Nolan SP. The measurement of intra-abdominal pressure as a criterion for abdominal re-exploration. Ann Surg. 1984;199(1):28–30.
10. Mullens W, Abrahams Z, Skouri HN, Francis GS, Taylor DO, Starling RC, Paganini E, Tang WH. Elevated intra-abdominal pressure in acute decompensated heart failure: a potential contributor to worsening renal function? J Am Coll Cardiol. 2008;51(3):300–6.
11. Cattermole GN, Leung PY, Ho GY, Lau PW, Chan CP, Chan SS, Smith BE, Graham CA, Rainer TH. The normal ranges of cardiovascular parameters measured using the ultrasonic cardiac output monitor. Physiol Rep. 2017;5(6):e13195.
12. Barnes GE, Laine GA, Giam PY, Smith EE, Granger HJ. Cardiovascular responses to elevation of intra-abdominal hydrostatic pressure. Am J Phys. 1985;248(2. Pt 2):R208–13.
13. Diebel LN, Wilson RF, Tagett MG, Kline RA. End-diastolic volume. A better indicator of preload in the critically ill. Archives of Surgery (Chicago, Ill: 1960). 1992;127(7):817–21.. discussion 821-812
14. Harman PK, Kron IL, McLachlan HD, Freedlender AE, Nolan SP. Elevated intra-abdominal pressure and renal function. Ann Surg. 1982;196(5):594–7.
15. Diebel LN, Dulchavsky SA, Wilson RF. Effect of increased intra-abdominal pressure on mesenteric arterial and intestinal mucosal blood flow. J Trauma. 1992;33(1):45–8.. discussion 48-49
16. Peoc'h K, Nuzzo A, Guedj K, Paugam C, Corcos O. Diagnosis biomarkers in acute intestinal ischemic injury: so close, yet so far. Clin Chem Lab Med. 2017;56(3):373–85.

17. Klingensmith NJ, Coopersmith CM. The gut as the motor of multiple organ dysfunction in critical illness. Crit Care Clin. 2016;32(2):203–12.
18. Mittal R, Coopersmith CM. Redefining the gut as the motor of critical illness. Trends Mol Med. 2014;20(4):214–23.
19. Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. Shock (Augusta, Ga). 2007;28(4):384–93.
20. Carrico CJ, Meakins JL, Marshall JC, Fry D, Maier RV. Multiple-organ-failure syndrome. Archives of surgery (Chicago, Ill: 1960). 1986;121(2):196–208.
21. Addington WR, Stephens RE, Phelipa MM, Widdicombe JG, Ockey RR. Intra-abdominal pressures during voluntary and reflex cough. Cough (London, England). 2008;4:2.
22. Cheatham ML, De Waele JJ, De Laet I, De Keulenaer B, Widder S, Kirkpatrick AW, Cresswell AB, Malbrain M, Bodnar Z, Mejia-Mantilla JH, et al. The impact of body position on intra-abdominal pressure measurement: a multicenter analysis. Crit Care Med. 2009;37(7):2187–90.
23. Shapiro MB, Jenkins DH, Schwab CW, Rotondo MF. Damage control: collective review. J Trauma. 2000;49(5):969–78.
24. Rotondo MF, Schwab CW, McGonigal MD, Phillips GR 3rd, Fruchterman TM, Kauder DR, Latenser BA, Angood PA. 'Damage control': an approach for improved survival in exsanguinating penetrating abdominal injury. J Trauma. 1993;35(3):375–82.. discussion 382-373
25. Griggs C, Butler K. Damage control and the open abdomen: challenges for the nonsurgical intensivist. J Intensive Care Med. 2016;31(9):567–76.
26. Ogilvie WH. Abdominal actinomycosis treated with sulphapyridine. Br Med J. 1940;2(4155):254–5.
27. Shaikh IA, Ballard-Wilson A, Yalamarthi S, Amin AI. Use of topical negative pressure in assisted abdominal closure does not lead to high incidence of enteric fistulae. Color Dis. 2010;12(9):931–4.
28. Fansler RF, Taheri P, Cullinane C, Sabates B, Flint LM. Polypropylene mesh closure of the complicated abdominal wound. Am J Surg. 1995;170(1):15–8.
29. Keramati M, Srivastava A, Sakabu S, Rumbolo P, Smock M, Pollack J, Troop B. The Wittmann patch is a temporary abdominal closure device after decompressive celiotomy for abdominal compartment syndrome following burn. Burns. 2008;34(4):493–7.
30. Cro C, George KJ, Donnelly J, Irwin ST, Gardiner KR. Vacuum assisted closure system in the management of enterocutaneous fistulae. Postgrad Med J. 2002;78(920):364–5.
31. Yuan Y, Ren J, Zhang W, Chen J, Li J. The effect of different temporary abdominal closure materials on the growth of granulation tissue after the open abdomen. J Trauma. 2011;71(4):961–5.
32. Deng Y, Ren J, Chen G, Li G, Wu X, Wang G, Gu G, Li J. Injectable in situ cross-linking chitosan-hyaluronic acid based hydrogels for abdominal tissue regeneration. Sci Rep. 2017;7(1):2699.
33. Hashizume R, Fujimoto KL, Hong Y, Amoroso NJ, Tobita K, Miki T, Keller BB, Sacks MS, Wagner WR. Morphological and mechanical characteristics of the reconstructed rat abdominal wall following use of a wet electrospun biodegradable polyurethane elastomer scaffold. Biomaterials. 2010;31(12):3253–65.
34. Stafiej P, Kung F, Thieme D, Czugala M, Kruse FE, Schubert DW, Fuchsluger TA. Adhesion and metabolic activity of human corneal cells on PCL based nanofiber matrices. Mater Sci Eng C Mater Biol Appl. 2017;71:764–70.
35. Gouveia PJ, Rosa S, Ricotti L, Abecasis B, Almeida HV, Monteiro L, Nunes J, Carvalho FS, Serra M, Luchkin S, et al. Flexible nanofilms coated with aligned piezoelectric microfibers preserve the contractility of cardiomyocytes. Biomaterials. 2017;139:213–28.
36. Stone HH, Strom PR, Mullins RJ. Management of the major coagulopathy with onset during laparotomy. Ann Surg. 1983;197(5):532–5.
37. Burch JM, Ortiz VB, Richardson RJ, Martin RR, Mattox KL, Jordan GL Jr. Abbreviated laparotomy and planned reoperation for critically injured patients. Ann Surg. 1992;215(5):476–83.. discussion 483-474

38. Roback JD, Caldwell S, Carson J, Davenport R, Drew MJ, Eder A, Fung M, Hamilton M, Hess JR, Luban N, et al. Evidence-based practice guidelines for plasma transfusion. Transfusion. 2010;50(6):1227–39.
39. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. J Trauma. 2003;54(6):1127–30.
40. Sondeen JL, Coppes VG, Holcomb JB. Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. J Trauma. 2003;54(5 Suppl):S110–7.
41. Malone DL, Hess JR, Fingerhut A. Massive transfusion practices around the globe and a suggestion for a common massive transfusion protocol. J Trauma. 2006;60(6 Suppl):S91–6.
42. Cohen MJ. Towards hemostatic resuscitation: the changing understanding of acute traumatic biology, massive bleeding, and damage-control resuscitation. Surg Clin North Am. 2012;92(4):877–91, viii.

Damage Control Resuscitation

2

Yu Chen and Xijing Zhang

Abstract

The concept of damage control resuscitation focuses on the reversing of the lethal trauma triad of coagulopathy, acidosis, and hypothermia in severe uncontrolled hemorrhage. Five key components of damage control resuscitation are permissive hypotension and restrictive fluid administration, hemostatic resuscitation, early hemorrhage control, correction of acidosis, and rewarming. Additional studies on the personalized resuscitations, such as individual blood product ratios, and targeting more accurately to those patients who can benefit most through additional high-quality prospective randomized intervention studies are needed.

Keywords

Trauma patient · Damage control resuscitation · Permissive hypotension · Restrictive fluid administration · Early hemorrhage control

2.1 Definition

Damage control resuscitation (DCR) is an optimal strategy for managing hemorrhaging trauma patients, incorporating several strategies to decrease mortality and morbidity.

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2.2 Background

The term "damage control" originates from World War II, a description of the US Navy's strategy to salvage sinking ships by operational status rather than repair all damage [1, 2]. Based on the hypothesis that surgical intervention may exacerbate imbalanced systemic inflammatory response during serious injury causing lethal consequences, the concept was adapted by medical practice, called damage control surgery (DCS), aiming to reduce this effect by minimizing the early surgical burden [3]. Other than surgery, stresses including hypotension, hypoxia, hypothermia, coagulopathy, acidosis, as well as infection may participate in this disordered immune response [4, 5]. As an extension from DCS, the concept of DCR includes early initiation of blood product transfusions over crystalloid fluid administration, permissive hypotension, and immediate hemorrhage control [6].

2.3 Pathophysiology

Understanding the physiologic sequelae of lethal triad of hypothermia, acidosis, and coagulopathy during trauma injury is essential to the application of DCR.

2.3.1 Hypothermia

Hypothermia is defined as a body core temperature less than 35 °C which is common in patients who have suffered from polytrauma. Depending on the symptoms, the clinical presentation of hypothermia is classified as three phases: mild (34–35 °C), moderate (32–34 °C), and severe (below 32 °C). Hypothermia is associated with a high mortality in trauma patients. Studies showed that when the core body temperatures drop below 32.8 °C, a nearly 100% mortality rate have been observed [2]. The causes of hypothermia are multiple:

1. Environmental reasons: at the scene of injury event, cold exposure during transport or/and in the emergency room will cause heat loss.
2. Iatrogenic treatment: the aggressive infusion of cold fluid.
3. Drug-induced factors: mild hypothermia is common during deep sedation or anesthesia.
4. Decreased oxygen supply: the anaerobic metabolism is less exothermic than aerobic metabolism, leading to less endogenous body heat production.

Hypothermia directly impairs blood coagulation by approximately 10% for each temperature decrease of one degree. The mechanisms may involve impeding platelet adhesion, reducing the enzymatic activity of thromboxane, dysregulating the coagulation factors, and interfering with fibrinolysis [7–9]. Hypothermia-associated coagulopathy may be covered up by the coagulation parameter testing by the

procedure of warming up of the blood sample prior to running the tests which should be considered in clinical practice for the trauma patients.

2.3.2 Acidosis

Acidosis is a condition in which there is an increased acidity in the blood and other tissues. For blood pH, the range is very narrow, from 7.35 to 7.45. Abnormal pH levels will affect the activity and stability of enzymatic, causing multiple tissue injury and organ failure. Decreased cardiac output, vasodilation, hypotension, and bradycardias may present when pH levels are below 7.2 [2]. Severe acidosis is highly related with the dysfunction of coagulation factors in the bleeding patient. The decrease of pH from 7.4 to 7.0 reduces the activity of factor VIIa and Xa/Va by 90% and 70%, respectively [2, 10, 11]. Acidosis may result from inadequate tissue perfusion, anaerobic metabolism, and overuse of normal saline for resuscitation, making the optimization of oxygen delivery very important in these situations [12].

2.3.3 Coagulopathy

Termed as acute traumatic coagulopathy (ATC), coagulopathy is common in the early stages of trauma patients. As an endogenous process, ATC causes bleeding morbidity and organ dysfunction [13, 14]. It is established that ATC firstly results from direct activation of the protein C pathway which is associated with the depletion of factors I, II, V, VII, VIII, IX, and X [14, 15]. Way of clotting dilution during the later resuscitation exacerbates coagulopathy further, which is recognized as dilutional resuscitation-related coagulopathy (DC) [16]. The increase rate of coagulopathy as the volume of intravenous fluid administration supports the view that clotting dilution exacerbates coagulopathy [17]. Combined acidosis and hypothermia further drives the severity of coagulopathy, causing unsalvageable situations [18]. Thus, quickly analyzing different aspects of the clotting cascade could help to understand individual component of coagulation dysfunction.

2.4 Damage Control Resuscitation

Focusing on the reversing of the lethal trauma triad of coagulopathy, acidosis, and hypothermia in trauma patients, DCR is consist of five pillars [1]:

1. Permissive hypotension and restrictive fluid administration
2. Hemostatic resuscitation
3. Early hemorrhage control
4. Correction of acidosis
5. Rewarming

2.4.1 Permissive Hypotension and Restrictive Fluid Administration

The concept of "permissive hypotension" refers to delay the initiation of fluid resuscitation and restrict the amount of fluids infusion in polytrauma patients. The purpose is to keep the blood pressure low enough to reduce the incidence and severity of dilutional coagulopathy while maintaining adequate organ perfusion. In practice, the systolic blood pressures (SBP) of 70–90 or a mean arterial pressure (MAP) of 50 mmHg is usually recommended.

It is not a new concept; in 1994, a semi-randomized controlled trial versus delayed fluid and standard/immediate resuscitation method demonstrated lower rates of SIRS, pulmonary edema, and thrombocytopenia in the delayed fluid resuscitation group [19]. Since then, different resuscitative protocols were argued [2]. The outcomes of this protocol are controversial; most subsequent trials have not found any benefit or harm; the approach may not be universal for all trauma patients [20–22]. A recent randomized controlled trial found that hemorrhagic shock patients with a MAP of 50 mmHg versus a MAP >65 mmHg used less blood products and IV fluids had lower early mortality (within the first 24 h) and higher mortality at 30 days [23]. For now, the effectiveness of permissive hypotension and restricted fluid administration is still inconclusive. For trauma patients, consideration should be taken in age, severity of injury, presence or absence of shock, and whether treatment occurred at a prehospital or in-hospital setting. Since low MAP reduces cerebral perfusion pressure (CPP) resulting in poor outcome in patients with severe traumatic brain injury (TBI), permissive hypotension is better avoided in this kind of patients.

2.4.2 Hemostatic Resuscitation

As mentioned before, ATC and DC are common in exsanguinating trauma patients. Recent varying protocols aim to administrate blood products early to help prevent trauma-related coagulopathy rather than waiting for the coagulation tests [1, 24, 25]. Timely administration of FFP and blood products was beneficial to reduce blood product use overall and improve outcomes [2, 5, 26–28]. Ideal transfusion ratio is still in dispute, different injury patterns may benefit from different regimes, but in practice, massive transfusion protocols (MTPs) should be considered as early as possible following any massive bleeding injury [29–31]. Coagulation tests with complete blood count (CBC), PT, PTT, fibrinogen, and platelet count can indicate component therapy. Advances in laboratory methods, such as thromboelastography-based protocols, can assess the function of the entire coagulation cascade in real time which has been suggested in some local MTPs [12, 32, 33].

2.4.3 Early Hemorrhage Control

Patients with hemorrhagic shock often continue to deteriorate despite resuscitative efforts until hemorrhage has been controlled [18]. Many major trauma protocols recommend that the patient should be delivered to an appropriate facility as quickly as possible [34, 35]. Before that, prehospital interventions are available to reduce bleeding [36]. Treatment options and their applicability have been fully discussed before [37]. Surgical hemorrhage control should be rapidly available whenever is possible. Procoagulant therapies remain controversial, including the administration of rFVIIa and tranexamic acid (TXA). The use of rFVIIa may reduce the transfusion requirement in bleeding patients, but the appropriate using time, requirements of additional blood components to function its effect, and subsequent thromboembolic complication remain debatable [38, 39]. Through blocking the lysine-binding sites on plasminogen, TXA can inhibit fibrinolysis [40] and reduce mortality rates by 15% in exsanguinating hemorrhage cases [19].

2.4.4 Correction of Acidosis

Metabolic acidosis may be intractable in the trauma patient until end-organ perfusion is restored by resuscitation, vasopressor support, and surgical control of hemorrhage. The treatment is aiming to reverse organ dysfunction through volume replacement, allowing the equilibrium of acid-base. Both bicarbonate and tris-hydroxymethyl aminomethane (THAM) can be used to directly reverse metabolic acidosis [41]. THAM does not produce excess sodium or by-product of CO2, it is suitable for patients with hypernatremia or concomitant respiratory acidosis [42]. However, no studies have shown any advantage about pharmacologic management of acidosis in the trauma setting, and there are currently no specific guidelines to address the specific reversal of acidosis in the trauma patient [29].

2.4.5 Rewarming

Hypothermia is one of the mortal factors in multiple trauma patients, but the benefits of rewarming are still unclear. Laboratory research have shown that mild hypothermia may increase the beneficial effect in selected situations, but this has not been proved in clinical practice; therefore adoption of rewarming in early treatment is still advocated [43]. Hypothermia is difficult to reverse than to prevent. When this happens, tissue perfusion would be diminished, which is associated with unfavorable prognosis in trauma patients. Since rewarming may increase vasodilation of vascular beds, it is advisable to rewarm the torso first to prevent worsening hypotension due to peripheral vasodilation. Depending on the rewarming rate and the injury severity, rewarming can be divided into three treatment strategies: passive external rewarming, active external rewarming, and active internal core rewarming.

- Passive external rewarming: techniques like remove wet clothing, use warm blankets, increase room temperature
- Active external rewarming: use forced-air warming systems and other heaters, like hot water bottles or heating pads
- Active internal core rewarming: methods like use warm and/or humidified air, warm intravenous fluids, peritoneal dialysis

Hemodialysis and cardiovascular bypass were proved effective. Invasive interventions are not suitable for trauma patient and better used in patients with stable clinical status. To prevent hypothermia from getting worse is the key point. Aggressive strategies should be considered if patients fail to respond to basic measures, and always be aware of occult ongoing hemorrhage [29].

2.5 Conclusions

The success of current DCR strategies is founded on the understanding of physiologic sequelae of lethal triad of hypothermia, acidosis, and coagulopathy during trauma injury. This time-dependent strategy addresses the early coagulopathy of trauma. Future evolution of the DCR concept may focus on personalized resuscitations, such as individual blood product ratios, targeting more accurately to those patients who can benefit most through additional high-quality prospective randomized intervention studies.

References

1. Ball CG. Damage control resuscitation: history, theory and technique. Can J Surg. 2014;57(1):55–60.
2. Duchesne JC, McSwain NE Jr, Cotton BA, Hunt JP, Dellavolpe J, Lafaro K, et al. Damage control resuscitation: the new face of damage control. J Trauma. 2010;69(4):976–90.
3. Giannoudi M, Harwood P. Damage control resuscitation: lessons learned. Eur J Trauma Emerg Surg. 2016;42(3):273–82.
4. Giannoudis PV, Dinopoulos H, Chalidis B, Hall GM. Surgical stress response. Injury. 2006;37(Suppl 5):S3–9.
5. Lasanianos NG, Kanakaris NK, Dimitriou R, Pape HC, Giannoudis PV. Second hit phenomenon: existing evidence of clinical implications. Injury. 2011;42(7):617–29.
6. Pohlman TH, Walsh M, Aversa J, Hutchison EM, Olsen KP, Reed RL. Damage control resuscitation. Blood Rev. 2015;29(4):251–62.
7. Wolberg AS, Meng ZH, Monroe DM 3rd, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. J Trauma. 2004;56(6):1221–8.
8. Beekley AC. Damage control resuscitation: a sensible approach to the exsanguinating surgical patient. Crit Care Med. 2008;36(7 Suppl):S267–74.
9. Scharbert G, Kalb ML, Essmeister R, Kozek-Langenecker SA. Mild and moderate hypothermia increases platelet aggregation induced by various agonists: a whole blood in vitro study. Platelets. 2010;21(1):44–8.
10. Martini WZ, Pusateri AE, Uscilowicz JM, Delgado AV, Holcomb JB. Independent contributions of hypothermia and acidosis to coagulopathy in swine. J Trauma. 2005;58(5):1002–9.

11. Martini WZ, Pusateri AE, Uscilowicz JM, Delgado AV, Holcomb JB. Independent contributions of hypothermia and acidosis to coagulopathy in swine. J Trauma. 2005;58(5):1002–9.. discussion 9-10.
12. Shapiro MB, Jenkins DH, Schwab CW, Rotondo MF. Damage control: collective review. J Trauma. 2000;49(5):969–78.
13. Cohen MJ, West M. Acute traumatic coagulopathy: from endogenous acute coagulopathy to systemic acquired coagulopathy and back. J Trauma. 2011;70(5 Suppl):S47–9.
14. Cohen MJ, Kutcher M, Redick B, Nelson M, Call M, Knudson MM, et al. Clinical and mechanistic drivers of acute traumatic coagulopathy. J Trauma Acute Care Surg. 2013;75(1 Suppl 1):S40–7.
15. Davenport R. Pathogenesis of acute traumatic coagulopathy. Transfusion. 2013;53(Suppl 1):23S–7S.
16. Tieu BH, Holcomb JB, Schreiber MA. Coagulopathy: its pathophysiology and treatment in the injured patient. World J Surg. 2007;31(5):1055–64.
17. Maegele M, Lefering R, Yucel N, Tjardes T, Rixen D, Paffrath T, et al. Early coagulopathy in multiple injury: an analysis from the German Trauma Registry on 8724 patients. Injury. 2007;38(3):298–304.
18. Fox CJ, Bowman JN. Advances in resuscitation in the setting of vascular injury. Perspect Vasc Surg Endovasc Ther. 2011;23(2):112–6.
19. Bickell WH, Wall MJ Jr, Pepe PE, Martin RR, Ginger VF, Allen MK, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. N Engl J Med. 1994;331(17):1105–9.
20. Dutton RP, Mackenzie CF, Scalea TM. Hypotensive resuscitation during active hemorrhage: impact on in-hospital mortality. J Trauma. 2002;52(6):1141–6.
21. Skarda DE, Mulier KE, George ME, Bellman GJ. Eight hours of hypotensive versus normotensive resuscitation in a porcine model of controlled hemorrhagic shock. Acad Emerg Med. 2008;15(9):845–52.
22. Turner J, Nicholl J, Webber L, Cox H, Dixon S, Yates D. A randomised controlled trial of prehospital intravenous fluid replacement therapy in serious trauma. Health Technol Assess. 2000;4(31):1–57.
23. Morrison CA, Carrick MM, Norman MA, Scott BG, Welsh FJ, Tsai P, et al. Hypotensive resuscitation strategy reduces transfusion requirements and severe postoperative coagulopathy in trauma patients with hemorrhagic shock: preliminary results of a randomized controlled trial. J Trauma. 2011;70(3):652–63.
24. Silverboard H, Aisiku I, Martin GS, Adams M, Rozycki G, Moss M. The role of acute blood transfusion in the development of acute respiratory distress syndrome in patients with severe trauma. J Trauma. 2005;59(3):717–23.
25. Dunne JR, Malone DL, Tracy JK, Napolitano LM. Allogenic blood transfusion in the first 24 hours after trauma is associated with increased systemic inflammatory response syndrome (SIRS) and death. Surg Infect. 2004;5(4):395–404.
26. Borgman MA, Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma. 2007;63(4):805–13.
27. Cotton BA, Gunter OL, Isbell J, Au BK, Robertson AM, Morris JA Jr, et al. Damage control hematology: the impact of a trauma exsanguination protocol on survival and blood product utilization. J Trauma. 2008;64(5):1177–82.. discussion 82-3.
28. Shaz BH, Dente CJ, Nicholas J, MacLeod JB, Young AN, Easley K, et al. Increased number of coagulation products in relationship to red blood cell products transfused improves mortality in trauma patients. Transfusion. 2010;50(2):493–500.
29. Kaafarani HM, Velmahos GC. Damage control resuscitation in trauma. Scand J Surg. 2014;103(2):81–8.
30. Undurraga Perl VJ, Leroux B, Cook MR, Watson J, Fair K, Martin DT, et al. Damage-control resuscitation and emergency laparotomy: Findings from the PROPPR study. J Trauma Acute Care Surg. 2016;80(4):568–74.. discussion 74-5.

31. Dente CJ, Shaz BH, Nicholas JM, Harris RS, Wyrzykowski AD, Patel S, et al. Improvements in early mortality and coagulopathy are sustained better in patients with blunt trauma after institution of a massive transfusion protocol in a civilian level I trauma center. J Trauma. 2009;66(6):1616–24.
32. Spinella PC, Strandenes G. The trauma hemostasis and oxygenation research network's remote damage control resuscitation symposium. Shock. 2014;41(Suppl 1):1–2.
33. Johansson PI, Sorensen AM, Larsen CF, Windelov NA, Stensballe J, Perner A, et al. Low hemorrhage-related mortality in trauma patients in a Level I trauma center employing transfusion packages and early thromboelastography-directed hemostatic resuscitation with plasma and platelets. Transfusion. 2013;53(12):3088–99.
34. Dutton RP. Haemostatic resuscitation. Br J Anaesth. 2012;109(Suppl 1):i39–46.
35. Khan S, Brohi K, Chana M, Raza I, Stanworth S, Gaarder C, et al. Hemostatic resuscitation is neither hemostatic nor resuscitative in trauma hemorrhage. J Trauma Acute Care Surg. 2014;76(3):561–7.. discussion 7-8.
36. Chesser TJ, Cross AM, Ward AJ. The use of pelvic binders in the emergent management of potential pelvic trauma. Injury. 2012;43(6):667–9.
37. van Oostendorp SE, Tan EC, Geeraedts LM Jr. Prehospital control of life-threatening truncal and junctional haemorrhage is the ultimate challenge in optimizing trauma care; a review of treatment options and their applicability in the civilian trauma setting. Scand J Trauma Resusc Emerg Med. 2016;24(1):110.
38. Geeraedts LM Jr, Kaasjager HA, van Vugt AB, Frolke JP. Exsanguination in trauma: a review of diagnostics and treatment options. Injury. 2009;40(1):11–20.
39. Stannard A, Eliason JL, Rasmussen TE. Resuscitative endovascular balloon occlusion of the aorta (REBOA) as an adjunct for hemorrhagic shock. J Trauma. 2011;71(6):1869–72.
40. Bailey AM, Baker SN, Weant KA. Tranexamic acid for trauma-related hemorrhage. Adv Emerg Nurs J. 2014;36(2):123–31.. quiz 32-3.
41. Jansen JO, Thomas R, Loudon MA, Brooks A. Damage control resuscitation for patients with major trauma. BMJ. 2009;338:b1778.
42. Hoste EA, Colpaert K, Vanholder RC, Lameire NH, De Waele JJ, Blot SI, et al. Sodium bicarbonate versus THAM in ICU patients with mild metabolic acidosis. J Nephrol. 2005;18(3):303–7.
43. Georgiou AP, Manara AR. Role of therapeutic hypothermia in improving outcome after traumatic brain injury: a systematic review. Br J Anaesth. 2013;110(3):357–67.

Perioperative Intestinal Injury: Etiology, Mechanism, and Prevention

3

Xiao-Dong Chen and Ke-Xuan Liu

Abstract

Perioperative organ injury is a severe and commonly encountered problem in surgical practice and has been drawing great attention from physicians and researchers. Under the philosophy of precision medicine and fast-track surgery, anesthesiologists have direct influence on patients' long-term outcomes by protecting important organs during perioperative period. This will reflect the evolvement of anesthesiology to perioperative medicine. There had been widespread concern over the mechanism and prevention of perioperative heart, brain, lung, and kidney injuries. Whereas the intestine is a luminal organ, research interests were often put to its digestive, absorbing, and excretory functions. In fact, intestines have much more functions than that mentioned above; intestine barrier has complex components, which can be easily affected by internal or external factors such as ischemia, hypoxia, infection, stress, or prolonged administration of antibiotics or immunosuppressants. Among these factors, intestinal ischemia is the most common cause of perioperative intestinal injury; this process not only occurs during the hypoperfusion stage but more importantly after blood supply was restored, namely, ischemia/reperfusion injury. The further intestinal injury caused by reperfusion and the consequent extraintestinal organ injuries were called second hit. The impaired intestinal mucosal barrier and subsequent translocation of intestinal bacteria and endotoxin can result in systemic inflammatory reaction syndrome. Here, we review the progress in the study of the mechanism, prevention, and treatment of perioperative intestinal injury (especially intestinal I/R injury), hoping to provide some useful information for clinical practice.

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Perioperative organ injury is a severe and commonly encountered problem in surgical practice and has been drawing great attention from physicians and researchers. Under the philosophy of precision medicine and fast-track surgery, anesthesiologists have direct influence on patients' long-term outcomes by protecting important organs during perioperative period. This will reflect the evolvement of anesthesiology to perioperative medicine.

There had been widespread concern over the mechanism and prevention of perioperative heart, brain, lung, and kidney injuries. Whereas the intestine is a luminal organ, research interests were often put to its digestive, absorbing, and excretory functions. In fact, the intestines have much more functions than that mentioned above; intestine barrier has complex components, which can be easily affected by internal or external factors such as ischemia, hypoxia, infections, stress, or prolonged administration of antibiotics or immunosuppressants. Among these factors, intestinal ischemia is the most common cause of perioperative intestinal injury; this process not only occurs during the hypoperfusion stage but more importantly after blood supply was restored, namely, ischemia/reperfusion injury. The further intestinal injury caused by reperfusion and the consequent extraintestinal organ injuries were called second hit. The impaired intestinal mucosal barrier and subsequent translocation of intestinal bacteria and endotoxin can result in systemic inflammatory reaction syndrome. Some hypovolemic shock patients died of severe lung complications even after blood pressure and urine volume had been recovered by anti-shock treatment. The reason was related to lung injury after intestinal I/R. Therefore, intestinal mucosal barrier damage usually occurs earlier than other organs; the intestinal tract is named "the triggering organ" and "the central organ of the surgical stress response." However, the importance of intestinal injury was often overlooked in clinical practice. Moreover, the mechanism of intestinal I/R injury remains unclear, and effective prevention and treatment is lacking. Here, we review the progress in the study of the mechanism, prevention, and treatment of perioperative intestinal injury (especially intestinal I/R injury) and extraintestinal organ damage, hoping to provide some useful information for clinical practice.

3.1 Etiology of Perioperative Intestinal Injury

3.1.1 Trauma and Shock

Traumatic-hemorrhagic shock is the main cause of death for people under the age of 45. It has been proved that if hemorrhagic shock could not be corrected in time, 51–61% of patients would eventually die of multiple organ failure (MOF) later, even though they survived the most dangerous phase of the pathophysiological changes. During hemorrhagic shock resuscitation, intestinal barrier function would be damaged because of the disproportionately decreased blood flow to the gut. The severe intestinal ischemia/reperfusion injury would be followed by bacterial translocation and endotoxemia, which led to systemic inflammation

via the intestine-liver-lung or intestine-lymph-lung path. The systemic inflammation would eventually lead to multiple organ failure (MOF) [1]. Even though the mesenteric artery can maintain blood flow relatively stable within a certain range of blood pressure, when blood pressure becomes under 40–45 mmHg, intestinal perfusion is compromised. Intestinal epithelial cellular injury in humans is detectable by 20 min of total mesenteric ischemia or within 60 min in the case of partial mesenteric ischemia [2]. Some vasoactive agents such as vasopressin, digoxin, phenylephrine, dopamine (high dose), and epinephrine (high dose) applied during shock resuscitation may also significantly reduce the intestinal blood supply [3].

3.1.2 Acute Pancreatitis

The intestinal injury caused by acute pancreatitis is mainly related to lowered mesenteric blood flow and decreased pHi of the intestinal mucosa. The small intestine may be damaged during severe acute pancreatitis, due to hypovolemia, splanchnic vasoconstriction, microcirculation disturbance associated with fluid loss in the third space, and finally ischemia/reperfusion injury [4]. SAP (severe acute pancreatitis) patients with abdominal compartment syndrome (intra-abdominal pressure IAP >20 mmHg) had an increased risk for intestinal ischemia. In SAP patients, release of inflammatory cytokines such as diamine oxidase and TNF-α leads to ischemia/reperfusion injury of gut mucosa which results in serious oxidative stress and activation of caspase-3 pathway and enhances epithelial cell apoptosis [5]. Elevated serum HMGB-1 (high-mobility group box 1) levels in SAP patients contribute to intestinal hyperpermeability and facilitate bacterial translocation and systemic inflammation [6].

3.1.3 Cardiac Surgery and Cardiopulmonary Bypass

De Silva et al. reviewed 348 patients by postmortem examination of 363 deaths from a total of 10,099 cardiac surgery performed over a 7-year period and found that among 52 patients whose actual primary cause of death was gastrointestinal (GI) origin, 45 patients died of intestinal ischemic injuries [7]. CPB leads to intestinal injury and is associated with hypoperfusion of the intestine. The exposure of blood to artificial materials used in the circuit and the altered blood flow and temperature may also activate inflammatory response. This can lead to ischemic damage in sensitive organs like the intestine [8]. It is reported that the endotoxin concentration in plasma can peak at 30 min after bypass and lasts for 20 h. Adamik et al. reported the serum endotoxin concentration elevated in 73% of patients with CPB time >90 min and 33% with CPB time <90 min, and their findings implied that intestinal ischemia/reperfusion injury occurred in all patients during cardiopulmonary bypass, but the magnitude of the intestinal damage depended on the duration of the cardiac bypass time [8].

3.1.4 Abdominal Aortic Aneurysm (AAA) Repair

Both open and endovascular (EVAR) AAA repair can cause bowel ischemia; the incidence rate is 1–3% for open and 0.5–3% for endovascular AAA repair, respectively. Ultee et al. reported that ruptured AAA was the most important determinant of postoperative bowel ischemia followed by open repair. Additional risk factors including patient characteristics were venerable age, female gender, hypertension, heart failure, and current smoking. Other risk factors from procedure features included unilateral interruption of the hypogastric artery, prolonged operative time, blood loss >1 L, and a distal anastomosis to the femoral artery. Bowel ischemia patients had a significantly higher perioperative mortality after intact as well as after ruptured AAA repair [9].

3.1.5 Liver Surgery

Intestinal injury may occur in liver operations, which is mainly related to hepatic portal occlusion. It was also reported that plasma endotoxin concentration was significantly related to hepatic portal occlusion. Li et al. retrospectively analyzed 1329 patients undergoing hepatectomy and found that the incidence of gastrointestinal complications reached 46.4% [10] and that long duration of anesthesia (odds ratio 2.51, $p < 0.001$) and the use of Pringle maneuver (odds ratio 1.37, $p = 0.007$) were independent risk factors of GI complications after hepatectomy. During the Pringle maneuver when vascular occlusion techniques are applied, the blood from the intestine and pancreas accumulated in the portal venous system leading to portal hypertension and gut congestion. Bowel wall edema may increase the intra-abdominal pressure and in turn deteriorate intestinal perfusion [11].

3.1.6 Intestinal Transplantation

Intestinal transplantation is a treatment option for irreversible intestinal failure. Both warm and cold ischemia phase insult the transplanted intestine directly for temporary interruption of blood supply. Then, the restoration of blood flow to the intestine graft causes oxidative stress and activates innate immunity leading to cell death [12].

3.1.7 Intestinal Diseases

Some disease directly insulting the intestine or intestinal vasculature can lead to intestinal ischemia, such as acute mesenteric thrombosis, mesenteric embolism, volvulus and neonatal necrotizing enterocolitis [13], etc. Symptoms of these diseases are often non-specific and can be subtle, resulting in a delay of correct diagnosis which decreases survival probability, especially when complicated with lung

injury and MOF. In infants with necrotizing enterocolitis, immature regulatory control of mesentery circulation makes the intestinal microvasculature vulnerable. The compromised vasculature increases circulation resistance and therefore decreases intestinal perfusion and may eventually progress to intestinal necrosis [14].

3.2 Mechanism of Perioperative Intestinal Injury

3.2.1 Intestine Barrier Function Impairment

The intestinal mucosal barrier consisted of mechanical barrier, immune barrier, chemical barrier, and microbiota barrier. Whether internal or external factors such as ischemia, hypoxia, infection, stress, etc. can impair these barrier functions, and these barrier function impairments often coexist with each other and have synergistic pathogenic effects.

3.2.1.1 Mechanical Barrier Function

Mechanical barrier function has a pivotal role in intestinal barrier functions. This role depends largely on the integrity of mucosal epithelial cells and tight junction between adjacent epithelial cells. Generally, the mechanical barrier function also includes intestine movement, intestinal epithelial cell cilia swing, and intestinal peristalsis, all of which can reduce the chance of pathogen adhesion to mucosal epithelial cells, preventing bacteria from staying in local intestinal mucosa for too long, just like intestinal self-cleaning. Mechanical barrier function impairment is characterized by increased intestinal mucosal permeability and epithelial cell damage such as cell necrosis, apoptosis, and ulceration. After exposure to the risk factors, blood redistribution occurs, and gut blood flow is drastically reduced in order to maintain important organ such as brain and heart blood supply, putting intestinal tract into ischemia, hypoxia, and acidosis state. Excessive oxyradical is produced by the gut, cellular metabolism disturbed by acidosis, and tissue injury induced. Simultaneously, extracellular calcium influx is triggered and intestinal mucosal epithelial edema aggravated. The epithelial cell membrane and intercellular junction rupture causes cell necrosis, ulceration, and increased intestinal mucosa permeability. On the other side, the intestinal tract can generate a large number of inflammatory mediators like nitric oxide, tumor necrosis factor, interleukin, interferon gamma, etc. under severe trauma, infection, and shock conditions. These inflammatory mediators interact with each other and form cascade reactions and then cause intestinal mucosa damage. Meanwhile, the reactive oxygen species (ROS) and inflammatory mediators produced by reperfusion subsequently can further damage the intestinal tract. When the body suffers trauma, chilliness, and pain, small intestine motility is inhibited through neurohumoral regulation resulting in prolonged bacterial retention and increased chance of colonization in the intestinal mucosa. Then, large amount of metabolite is produced by the implanted bacteria, and the gut mucosa structure damaged.

Reactive oxygen species are by-products of normal metabolism of cells, which can be beneficial to tissues at low and moderate doses by assisting wound healing, pathogen elimination, and tissue repair. Under pathological conditions like ischemia/reperfusion, excessive ROS is generated and causes oxidative damage to proteins and DNA and increased lipid peroxidation, leading to altered cell growth, differentiation, and apoptosis. Reperfusion-caused tissue damage is primary due to the reentry of oxygen rather than ischemia itself. It is explained by some researcher that ATP is metabolized into hypoxanthine during the ischemia period; when reperfusion starts, oxygen reacts with hypoxanthine to form xanthine and superoxide anion, large amount of oxygen-free radicals. Reperfusion enhances the ischemia injury by generating excessive ROS and accumulating activated neutrophils [15, 16].

Liu et al. built the animal model of intestinal ischemia/reperfusion injury by temporarily clamping and reopening the superior mesenchymal artery (SMA) in rats. Through animal experiment they found large amount of intestinal epithelial cell necrosis and apoptosis after intestine ischemia/reperfusion, and that intestinal injury could be improved by inhibiting cell apoptosis [17]. Based on these researches, it can be deduced that searching for important target of intestinal mucosal epithelial cell apoptosis regulation should be the key to prevent and treat intestinal I/R injury. Then, Wen et al. carried out a series of studies using the rat SMA occlusion/reopen model and demonstrated that aldose reductase, alpha-2 adrenergic receptor, and JAK2/STAT3 signaling were important molecules regulating intestinal mucosal epithelial cell apoptosis induced by intestinal ischemia/reperfusion [18]. In addition, Liu KX and his colleague described the microRNA expression profile during intestinal ischemia/reperfusion in rats for the first time and found that 19 microRNA expressions changed after I/R in intestinal mucosa, 1 upregulated and 18 downregulated including miRNA378. Through further studies of its function, they found upregulation of miRNA378 could reduce intestinal mucosal cell apoptosis and relieve gut injury [19].

Recent studies have shown that cell ischemic injury can cause not only apoptosis and necrosis but also another form of cell death, necroptosis, which morphologically looks like necrosis and apoptosis combined. Necroptosis is regulated by a series of signal pathways and metabolic mechanisms, except apoptosis-regulating factors such as caspase family, and is energy consuming. Wen et al. discovered that administrating necroptosis inhibitor Nec-1 could relieve gut ischemia/reperfusion injury in rats, and blocking both the apoptosis and necroptosis by using Nec-1 and Z-VAD (pan-caspase inhibitor) combined provided better intestinal protection. Our studies demonstrated that necroptosis played an important role in the mechanism of intestinal epithelial cell death after gut ischemia [17].

Tight junctions (TJ) are located at the apical lateral region of adjacent intestinal epithelial cells and composed of a group of transmembrane proteins such as claudins and occludins, as well as peripheral membrane proteins zonula occludens proteins (ZO-1-3). They play the major part of mechanical function of intestinal barriers by maintaining epithelial integrity, cellular polarity, and homeostasis and regulating the permeability of paracellular spaces in the epithelial mucosa [20–22]. Takizawa

et al. reported that damage of intestinal mucosa by intestinal I/R injury occurred with the start of reperfusion in a manner dependent on the severity of the lesion. Claudin-2 is an important factor for TJ construction and its remodeling. Claudin-4 regulates paracellular permeability in the intestine during intestinal ischemia/reperfusion [23, 24]. Chi et al. demonstrated that in a liver transplant-induced intestinal I/R model, occludin and ZO-1 expression in intestinal epithelia decreased, accompanied by elevated concentrations of D-LA, DAO, and FABP2 in serum, which suggested a compromised TJ structure leading to the epithelial barrier dysfunction [20]. Paracellular permeability across intestinal epithelium is largely regulated by the tight junctions. Increased intestinal permeability results in leakage of bacteria, microbial products, or other antigens from the intestinal lumen into the lamina propria to initiate or exacerbate an inflammatory response [21].

3.2.2 Immune Barrier Function

The intestinal immune barrier mainly consists of intestinal plasma cell secretory immunoglobulin (S-IgA), gut-associated lymphoid tissue (GALT), and liver defense function (gut-liver axis). These components work together to prevent pathogen invasion and injury to the gut and body through humoral and cellular immunity.

3.2.2.1 Humoral Immune Function Impairment

S-IgA is the main immune defense against luminal pathogens at intestinal mucosal surfaces. This defensive effect may be weakened by significantly inhibited intestinal S-IgA production, including lowered S-IgA content, S-IgA synthesizing plasma cell loss or dysfunction, and decreased S-IgA binding capacity to Gram-negative bacteria. The gut cannot maintain its resistance to bacterial colonization due to insufficient S-IgA production, and then intestinal bacteria translocation occurs. Zhang et al. reported that intestinal I/R resulted in impaired class switch recombination (CSR) of IgM B^+ cells, a key biological process involved in mucosal IgA synthesis, in Peyer's patches (PPs) and decreased secretory IgA concentration in the gut lumen 2 h after reperfusion [25]. Then they employed transforming growth factor β (TGF-β) to activate IgA CSR and promote IgA production in the intestinal tract of rats and demonstrated that TGF-β could improve gut IgA secretion and protect intestinal mucosal epithelial integrity against ischemia/reperfusion injury and lower the 24-h death rate of intestinal I/R rats [26]. S-IgA also has anti-inflammatory activities. It is reported that S-IgA can inhibit enterocyte apoptosis induced by ischemia/reperfusion and maintain mucosal integrity [27].

Natural immunoglobulin (Ig) M has been identified as one of contributors to I/R injury. Non-muscle myosin type II (NM-II) heavy chain A and C have been recognized as self-targets of natural IgM and IR injury in the small intestine of mice [28]. Ischemia-mediated aggregation of the actin cytoskeleton leads to the deposition of natural IgM and the activation of complement [29]. Reperfusion of the ischemic tissues shows an acute inflammatory response activated by natural IgM binding to ischemia-specific self-antigens, which are non-muscle myosin heavy chains type II

(NM-II) heavy chain subtype A and C. The natural IgM-antigen complexes combine with mannan-binding lectin (MBL) and activate the complement [28], a story part of the innate immune system which recognizes self-antigen and initiates inflammatory responses the way they encounter pathogens. Neoantigens or modified epitopes presented on the cell surface in ischemic tissues may also trigger complement activation via natural IgM deposition. Three pathways leading to the activation of the complement system have been identified: the classical, the lectin, and the alternative pathways. Each is activated by different initiators but all converge on C3 activation, which is followed by a common cascade. Lectin complement pathway operates immediately downstream of the natural IgM-ischemic antigen interaction during intestinal I/R. The classical complement pathway also appears to interact with the natural IgM-ischemic antigen immunocomplex. But the alternative complement pathway was not seen involved in I/R injury induction in the mice model [28]. Intracellular ROS formation and lipid peroxidation leads to an innate autoimmune response by natural IgM and complement [30]. It is reported that activation of complement C5a can deteriorate intestinal I/R-induced lung injury through autophagy-mediated alveolar macrophage apoptosis [31]. Inhibiting C5a activation significantly reduces the inflammatory cells such as polymorphonuclear leukocyte and macrophages near the injured site, increases proliferation of epithelial cells in the villi, and prevents further damage [32]. In addition, it is also reported that the ischemic tissue expresses a chemokine CXCL13 that attracts B cell, and accumulation of B cells contributes to the intestinal ischemia/reperfusion injury through an antibody-independent way [33].

Paneth cells are located at the base of the crypts of Lieberkühn (intestinal gland) and participate in the innate immune response by secreting several antimicrobial compounds including α-defensins and lysozyme into the gut lumen when exposed to bacteria or bacterial antigens. Because of its active secretory function, Paneth cell is vulnerable to endoplasmic reticulum (ER) stress which arises from a series of conditions including ischemia. Intestinal I/R results in activation of the unfolded protein response may also lead to Paneth cell apoptosis. Paneth cell dysfunction increases bacterial translocation and systemic inflammation in rats simultaneously with physical intestinal damage compared with rats with normal Paneth cell function [34].

3.2.2.2 Cellular Immune Function Impairment

Gut-associated lymphoid tissue (GALT) plays a central role in the systemic mucosal immune system; the reduction of GALT mass and function may disturb mucosal immunity. Lack of oral intake after gut I/R contributes to the reduction in GALT cell number [35] and also leads to intestinal mucosal T-cell dysfunction; hence, the mucosal resistance to infection is lowered. The goblet cell is insulted at the same time; its secretion of mucin is decreased, reducing the non-specific barrier function of gut mucosa. Severe infection, inflammation, and shock not only cause systemic immune dysfunction but also reduce lymphocyte in lamina propria and damage GALT. In addition, they also lower S-IgA secretion and induce Kupffer cell apoptosis and then destroy the intestinal mucosal immune barrier. Watson et al. used

anti-thymocyte globulin (ATG) to deplete T cells in a ischemia/reperfusion mice model and found that the MPO and proinflammatory factors such as tumor necrosis factor-α, interferon-γ, and interleukin-2 decreased with less local T-lymphocyte infiltration, and the epithelial cell apoptosis was also inhibited [36]. Regulatory T cells (Tregs), expressing CD4 and CD25, are a subset of T cells that modulate the immune system, maintain tolerance to self-antigen, and downregulate induction and proliferation of effector T cells. Partial depletion of Treg enhances secretion of tumor necrosis factor-α, interferon-γ, and interleukin (IL)-4 and increases intestinal permeability, while adapted transfer of Treg can reverse this effect [37].

Mast cells (MCs) constitute 2–3% of lamina propria cells; as a part of the immune system of the gut, they serve as first responders to invading pathogens and cellular stress signals. During intestinal ischemia/reperfusion, activated mast cells move to the tip of villus tip area and degranulate, releasing numerous inflammatory cytokines and causing tissue injury [38]. Intestinal ischemia/reperfusion may also activate macrophages, which then release cytokines, enhance myeloperoxidase activity, promote ROS production, and cause epithelial cell death [39]. Liu et al. reported that intestinal I/R injury caused a switch from M2 to M1 macrophages. Recombinant *Trichinella spiralis* cathepsin B-like protein (rTsCPB) could significantly relieve mucosal injury and improve intestinal function by promoting M1 to M2 macrophage phenotype transition. Inhibiting M1 to M2 transition could reverse the protective effects of rTsCPB. This study provided a potential choice of therapeutic agent that might be used in intestinal I/R injury [40]. Kupffer cells, the hepatic specific macrophages, are important sources of amplification of proinflammatory cytokine release such as TNF-α and IL-6 after intestinal ischemia, which in return aggravate intestinal injury [41].

3.2.3 Microbiota Barrier

The intestinal tract harbors the majority of microorganisms of the human body, up to 78% of total microbial populations, and 95% of the microbiota in gut are anaerobic bacteria. The anaerobic bacteria attach to the specific receptors on the epithelial cell surface forming microbial membrane structures, and they can restrain the pathogen colonization and proliferation and promote intestinal peristalsis and mucus flow. The intestinal resident bacteria and the host's intestine spatial microstructure are interdependent and interactive with each other; they form a relatively stable, multilayer microecosystem in the gut lumen, biologically antagonistic to pathogens. Microbiota activate the immune system in the gut and enhance the secretion of S-IgA and IgM. A relatively large portion of microbiota are coated with S-IgA, forming microbiota S-IgA complex, and help to maintain gut homeostasis. Free S-IgA upregulates expression of TNF-α, IL-8, and polymeric immunoglobulin receptor, whereas microbiota-complexed S-IgA ameliorates this effect [42]. When the balance between host and microflora or among different groups of microbiota was interrupted by exogenic factors, the intestinal biological barrier is impaired through the following mechanisms: First, followed by mechanical gut injury, the

intraluminal microflora enters submucosal tissue through the intestinal mucosa with increased permeability, leading to bacteria translocation, breaking the equilibrium of intestinal microbiota and the balance of gut microecosystem and then damaging intestinal biological barrier. Second, diminished bile secretion and intestinal-hepatic circulation disorder result in intestinal dysfunction and overgrowth of intestinal bacteria. Third, depressed peristalsis or oxygen uptake can lower intestinal metabolic rate and cause intestinal flora imbalance, G-bacteria overgrowth, and excessive production of endotoxin which can damage the intestinal mucosa.

Hoehn et al. reported that intestinal ischemia/reperfusion lead to bacterial overgrowth in the jejunum lumen and increases concentration of ceramide in the jejunal villi vasculature as well as the cell membrane of proliferated bacteria [43]. Ceramide has been demonstrated to be related to intestinal epithelial cell apoptosis, and antioxidative anesthetics like propofol and *Ginkgo biloba* extract can inhibit gut epithelium apoptosis via reducing ceramide production [44, 45]. Wang et al. used DGGE fingerprints to investigate the gut microbiota pattern changes after intestinal ischemia/reperfusion and figured out that *Escherichia coli* proliferation accompanied by *Lachnospiraceae* and *Lactobacilli* reduction was the characteristics of dysbiosis followed by intestinal I/R injury. Pattern changes of these three groups of bacteria are associated with increased proinflammatory factor secretion and pathogen adherence as well as decreased antimicrobial substances and tight junction assembly [46].

3.2.4 Chemical Barrier

The intestinal chemical barrier consists of mucus, digestive juice, and antimicrobial substance produced by intestinal microbiota. These components can dilute toxin, kill pathogenic bacteria, and combine with and limit the absorption of endotoxin. Mucus is mainly secreted by goblet cells, and intestinal ischemia/reperfusion significantly decreases goblet cell numbers in animal models [47]. In patients with severe infection or trauma, their intestinal mucosal renewal and repair ability is reduced by lacking stimulation of food and digestive hormone due to fasting. Meanwhile, the secretion of gastric acid, bile, lysozyme, and mucopolysaccharide decreased, weakening the chemical bactericidal ability of digestive juice. In some patient who underwent continuous gastrointestinal decompression, the bactericidal, toxin dilution and endotoxin combination ability decreased significantly due to gastric acid, bile, and pancreatic juice loss. In addition, drugs used to lower stomach acidity to prevent stress ulcer in critically ill patients can also weaken the chemical barrier function. It has been demonstrated that intestinal ischemia/reperfusion can cause gastrin dysfunction, inhibit basal acid secretion and weaken the acid secretory response to pentagastrin, and induce accumulation of alkalized gastric fluid rich in glucose, protein, and bicarbonate [48]. Liu et al. reported that elevated serum gastrin concentration could ameliorate epithelial disruption, decrease disintegration of lamina propria, reduce proinflammatory factor production that inhibits apoptosis, and lower mortality in intestinal I/R rat models [49]. Mucin, the major protein of mucus, is disrupted during the early phase of ischemia [50]. I/R insult leads to oxidative modification

and extrusion of the mucus layer into the intestinal lumen, and the mucus is further degraded by pancreatic proteases; the thinned and degraded mucus layer allows pancreatic proteases and bacteria to cross the epithelium line and enter the submucosal space and triggers inflammatory response [51]. Qin et al. reported that treatment of the gut with the mucolytic NAC during ischemia could enhance mucosal permeability and aggravate gut injury and that the presence of digestive proteases by themselves did not exacerbate gut injury, but in combination with NAC, they induced an even greater increase in intestinal permeability and injury [52]. Grootjans et al. reported that intestinal ischemia lead to mucus layer detachment in the colon and bacteria penetration to the mucosa, while increasing secretory activity of goblet cells could expulse the bacteria out of the crypts and restore mucus layer [53].

3.2.5 Remote Organ Damage Due to Intestinal Injury

Impaired intestinal barrier function leads to bacteria and endotoxin translocation and activates the monocyte/macrophage system to release a large number of inflammatory mediators and cytokines, resulting in systemic inflammation and even multiple organ dysfunction syndrome and multiple organ failure. Lung and heart injuries after intestinal I/R, the second hit, are an important prognostic factor for patient survival. Patients who underwent cardiopulmonary bypass or abdominal aortic aneurysm surgery often experienced cognitive dysfunction, suggesting that they may have concomitant brain injury. To clarify whether their brain injury was associated to intestinal I/R or not, Zhou and Liu et al. built an intestinal I/R injury model in rats and found that intestinal I/R could induce brain edema and cerebral cortex and hippocampus damage in rats, accompanied by reduced memory and spatial learning capacity, as well as decreased survival rate of the animals. Further studies demonstrated that microglia activation aggravated inflammation response and oxidative stress injury in the brain tissue and promote neuronal cell apoptosis. It may be the major part of the mechanism of intestinal I/R-induced brain damage and memory dysfunction. Liu et al. investigations suggest that close attention should be given to cognitive dysfunction manifestations of patients with intestine I/R risk factors [54]. In addition, beta-defensin-2 is one of the endogenous protective factors in the human body, and it is one of the main factors for the body to resist exogenous stimuli. Liu et al. found that I/R upregulated both gene and protein expression of beta-defensin-2 in rats. The expression of beta-defensin-2 was related to the activation of inflammatory cytokines in the lung, and when its expression was upregulated in the lung, it could significantly improve pulmonary injury caused by intestinal I/R [55].

3.3 Prevention and Treatment

Many explorations of the strategy to prevent intestinal barrier injury have been made in the recent years as the knowledge on intestinal mucosal barrier functions deepens. Numerous regimen and therapies have been reported to be efficacious in

preventing or treating intestinal ischemia/reperfusion injury in experimental animal studies. The vast majority of animal model used in these studies was the mouse/rat model established by temporarily clamping and reopening of the superior mesenteric artery. However, the treatment options available for intestinal ischemia/reperfusion injury in the clinical settings are still limited.

3.3.1 Early Enteral Nutrition

Early enteral nutrition (EN) support is the primary measure to protect the intestinal mucosal barrier function; it can prevent intestinal mucosal atrophy and reduce intestinal bacterial translocation. It also stimulates gastrointestinal hormone release, promotes gut peristalsis, and brings quicker recovery of intestinal barrier function. Enteral nutrition with addition of arginine, polyunsaturated fatty acids, nucleotides, vitamin A, and glutamine facilitates this process. Lin et al. reported that both conventional and lipid-rich EN via gavage increased the intestinal motility and the intestinal mucosal tight junction protein expression and decreased the serum I-FABP levels and reduced systemic inflammatory response after intestinal I/R injury in rats, while lipid-rich EN conferred better effects in controlling intestinal inflammation and improving mucosal barrier function than conventional EN did [56]. Wu et al. demonstrated that 20% dose of enteral nutrition had a significantly better protective effect than total parenteral nutrition did on intestinal barrier function in rat II/R model [57].

3.3.1.1 Glutamine (Gln)

Glutamine is the main energy source of intestinal epithelium and essential nutrient for intestinal mucosa metabolism and regeneration. It is also the precursor of reduced glutathione, an important component of the cellular antioxidant system. Under severe stress conditions, glutamine demand will exceed its production; deficiency of Gln will lead to atrophy of intestinal mucosa, thinning of villi, and impaired intestinal barrier function. Extra-enteral nutrition with proper supplementation of glutamine or enteral nutrition can increase intestinal villus height, reduce intestinal mucosal permeability and enhance intestinal immunity, prevent bacterial translocation, and then maintain intestinal mucosal barrier function. Shu et al. reported that rats fed with vegan chow with 3% Gln supplementation exerted beneficial anti-inflammatory effect and improved morphological changes in the gut mucosa after intestinal ischemia/reperfusion [58]. It is also found that peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily of transcription factors which is highly expressed in intestinal epithelium, played a pivotal role in enteral Gln's protection to the postischemic gut [59].

3.3.1.2 Growth Factors

Epidermal growth factor (EGF) plays an important role in the growth, differentiation, and tissue repair of intestinal epithelial cells. It can increase the synthesis of glutamine in the body and promote the absorption and utilization of glutamine in the

intestine and has a synergistic effect with glutamine on the intestinal tract. It has been found that EGF can reduce intestinal bacterial translocation and lower mortality rate in patient subject to total parenteral nutrition. Insulin-like growth factor (IGF) is synthesized by liver cells and contributes to cell division. Animal studies have shown that it can increase DNA and protein synthesis in intestinal mucosal cells, restrain intestinal mucosal atrophy and barrier destruction, and reduce the mortality rate of infection.

3.3.2 Anti-oxyradical Agent

The intestine is rich in xanthine oxidase (XOD), which produces oxygen-free radicals (OFR) under hypoxia or ischemic conditions. Excessive OFR causes intestinal mucosa damage but can be antagonized by exogenous or exogenous OFR scavengers. Selenium is an essential trace element known to have an antioxidant effect as a critical cofactor for the function of glutathione peroxidase, which is involved in the oxidation of glutathione. Kim et al. reported that selenium treatment might protect the intestine by increasing glutathione peroxidase activity, reducing lipid peroxidation, and downregulating the NF-κB pathway during intestinal I/R injury in rats [60]. Trapidil is a phosphodiesterase and platelet-derived growth factor inhibitor. Colak et al. demonstrated that trapidil treatment could protect intestinal barrier function in rat intestinal I/R model via inhibiting lipid peroxidation, improving nitric oxide metabolism, and suppressing thromboxane A2 and proinflammatory cytokine production [61, 62]. Melatonin is an endogenous hormone secreted by the pineal gland and has strong anti-oxidative effect; pretreating melatonin or its natural intermediate product, N-acetylserotonin, can attenuate intestinal I/R-induced intestinal and lung injuries [63–65]. Pyruvate has been proved to be a potent ROS scavenger, directly neutralizing peroxides and peroxynitrite and also scavenging hydroxyl radicals. Pyruvate-peritoneal dialysis solution (PDS) following intravenous resuscitation improves intestinal barrier function in rats with hemorrhagic shock through eliminating free oxygen radicals, reducing neutrophil infiltration, and inhibiting inflammatory response [66, 67]. Resveratrol is a natural phytoalexin found in dietary sources such as grapes, plums, peanuts, and wine. Accumulating evidence indicates that resveratrol may have therapeutic potential for intestinal I/R injury by its antioxidant, anti-inflammatory, and antiapoptotic properties [68]. Resveratrol treatment yields effects of protecting myenteric neurons and limiting enteric glial cell proliferation in the ileum, alleviating lung injury via suppressing mast cell degranulation in intestinal I/R rats [69, 70].

3.3.3 Traditional Chinese Medicine

The traditional Chinese medicine (TCM) differentiation theory attributes microcirculation disorders in early intestinal ischemia/reperfusion to poor blood supply and blood stasis, and many medications were developed under this theory. Xuesaitong

injections, whose major component is panax notoginseng saponins, the active ingredient of notoginseng plant, are widely used prior to clinical procedures. Xu et al. reported that intraperitoneal injection of Xuesaitong once a day for a week prior to ischemia improved gut peristalsis and reduced intestinal mucosal apoptosis after reperfusion in the IRI rat model [71]. Jiang et al. demonstrated that ginsenoside Rb1 could alleviate lung histological injury, suppress inflammatory responses, and reduce tissue edema by activating nuclear factor erythroid 2-related factor-2 (Nrf2)/heme oxygenase-1(HO-1) pathway in mice model of intestinal I/R-induced lung injury [72]. Qinghuobaiduyin (QHBDY) is a traditional Chinese medicine that has been used to treat burn patients for more than 20 years. Zhu et al. demonstrated QHBDY reduced intestinal epithelial cell apoptosis following burn at both animal and cellular levels [73]. Mo et al. reported that osthole, a component of a traditional Chinese medicine fructus cnidii extract, could inhibit inflammatory response and oxidative stress and attenuate the lung injury induced by II/R in rats [74].

3.3.4 Anesthetics

It has been demonstrated that commonly used anesthetics, like propofol, remifentanil, and dexmedetomidine, can attenuate intestinal I/R-induced intestinal injury. The gut protection effect of these drugs might be related to their antioxidant properties reducing lipid peroxidation and attenuation of apoptosis of intestinal epithelial cells [44, 45, 75, 76]. Gan et al. reported that propofol could inhibit intestinal I/R--induced NADPH oxidase overexpression and suppress ROS-mediated mast cell degranulation [77]. The suppression of ROS and mast cell activation by propofol can also alleviate lung injury induced by intestinal I/R [78]. Gan et al. found that rats exposed to 2.3% sevoflurane for 1 h for 3 subsequent days prior to ischemia/reperfusion procedure showed reduced inflammation and increased SOD activities in the intestinal mucosa as well as improved postischemic survival rate possibly by inhibiting oxidative stress in a mast cell-independent way [79]. Liu et al. demonstrated that pretreatment with 0.5 MAC sevoflurane inhalation in intestinal I/R rat model attenuated gut injury by inhibiting intestinal mucosal epithelial apoptosis via activation of the PI3K/Akt pathway [80]. Kim et al. showed that 4 h of 1MAC isoflurane inhalation after intestinal ischemia led to inhibited epithelial cell apoptosis, alleviated intestinal injury, and improved renal and hepatic dysfunction by induction of intestinal epithelial TGF-beta1 [81]. Shen et al. conducted remifentanil pretreatment in rat model using three cycles of 5 min of remifentanil infusion alternating with 5 min of normal saline before ischemia and found that remifentanil pretreatment inhibited intestinal epithelial cell apoptosis and ameliorated histological injury. The protective effect was dose independent within the range of 0.2–1 mg kg^{-1} min^{-1} RF and was achieved by acting via intestinal delta- and mu-opioid receptors [75]. Dexmedetomidine is a potent and highly selective α2 adrenoreceptor agonist widely used in clinical anesthesia. It has also been demonstrated to have anti-inflammatory and organ protective effects. Zhang et al. reported that dexmedetomidine administration before ischemia at a dose of 5 μg kg^{-} h^{-} in rats, which

is equal to 0.8 μg kg^{-} h^{-} in humans, attenuated intestinal ischemia/reperfusion injury partly by inhibiting inflammatory response and intestinal mucosal epithelial apoptosis via activating the α2-adrenoceptors [76].

3.3.5 Antiendotoxin Therapy

Endotoxins are the major components of the outer membrane of most Gram-negative bacteria [82]. Endotoxin can be removed by peritoneal lavage, hemodialysis, or activated carbon adsorption. Whole-bowel irrigation can reduce the number of intestinal flora and drastically decrease the amount of the absorbable endotoxin pool in the gut. Selective decontamination of digestive tract (SDD) can be used to inhibit the production of intestinal endotoxin by diminishing the number of Gram-negative bacilli in the intestinal tract. The widely used SDD regimen is PTA (polymyxin E, tobramycin, and amphotericin B) in clinical settings. Lactulose, a nontoxic synthetic disaccharide, reduces endotoxin absorption by reducing or altering the intestinal flora. Oral application of lactulose can prevent or eliminate systemic endotoxemia.

3.3.6 Ischemic Preconditioning/Postconditioning

Ischemic preconditioning is a physiologic mechanism by which tissues and organs exposed to a short period of ischemia/reperfusion(I/R) prior can develop resistance to ischemic insults and significantly attenuate intestinal injury by inhibiting the neutrophil-endothelial adhesion cascade via reducing ICAM-1 and VCAM-1 expression and TNF-α-induced NF-κB signaling pathway [83]. However, organ ischemia episodes are often unpredictable, and the actual treatment can only be applied afterward. Considering the limitations of preconditioning, here comes a more clinically feasible concept of ischemic postconditioning that reperfusion or ischemia treatment is performed immediately after the initial longer period of ischemia/reperfusion insult. It was found that myocardial I/R damage can be significantly reduced by postconditioning treatment. Liu et al. have done a series of investigations to explore the gut protection effect and mechanism of ischemic postconditioning in intestinal I/R rats. Their discoveries are as follows. First, ischemic preconditioning and postconditioning have similar protective effect against intestinal and lung injury induced by intestinal I/R in rats, and their effects can be synergistic. Second, if the postconditioning treatment were not applied until 3 min after reperfusion (i.e., delayed treatment), the protective effect of postconditioning disappeared, suggesting that the protective effect have a time window and ischemic postconditioning treatment should be carried out within this period in clinical practice. Third, both ischemic preconditioning and postconditioning play a role in intestinal protection by upregulating the expression of aldose reductase and inhibiting intestinal mucosal cell apoptosis, and aldose reductase is the common target. Fourth, postconditioning treatment inhibits intestinal mucosal cell apoptosis by inhibiting

JAK2/STAT3 pathway [18, 84, 85]. Postconditioning also downregulates the transcription of Toll-like receptor 4 (TLR-4) mRNA, reduces protein expression of high-mobility group box-1 (HMGB-1) and TLR-4 in the intestinal mucosa, and inhibits cell apoptosis and inflammatory response [86]. Besides the unpredictability of ischemic episode, preconditioning itself has another limitation. It might deteriorate organ function or cause complications, such as plaque embolization, especially when arteries are intermittently occluded. Recently, a more clinically applicable stimulus is afforded by remote ischemic preconditioning (RIPC), which has been noted that a brief I/R in distant tissues, usually skeletal muscle, may bring the same protection effect in the heart. However, this method had not been reported to improve the long-term outcome of the patient, neither its role in intestinal I/R injury had been demonstrated. Li et al. evaluated the effect of limb RIPC in providing intestinal and pulmonary protection after elective open infrarenal abdominal aortic aneurysm (AAA) repair in a single-center, prospective, double-blinded, randomized, parallel-controlled trial. During the trial, it was demonstrated that intermittent upper limb ischemia as a RIPC stimulus conferred potential intestinal and pulmonary protection during elective open infrarenal AAA repair and significantly improved bowel and lung function while reducing postoperative hospital stay at ICU [87].

3.3.7 Stem Cell

Stem cells are undifferentiated biological cells and have the potential to differentiate into specified cells in specific organs and tissues. In humans, stem cells can be divided into two broad types: embryonic stem cells and adult stem cells. Stem cell therapy has gained much attention from researcher and physicians; many studies have shown encouraging effect from stem cell treatment. Because of ethical concerns, adult stem cells are the overwhelming majority of stem cell sources in these studies. Bone marrow stem cells can migrate, colonize, and differentiate into specific injured organs in vivo to accelerate the repair of damaged tissues. In many animal experiments and clinical studies, exogenous stem cell transplantation had been used in the treatment of intestinal injury. Shen et al. reported that bone marrow transplantation protected intestinal mucosal barrier function against intestinal I/R injury by inhibiting ZO-1 downregulation and tight junction disruption [88]. Adipose-derived mesenchymal stem cells (ASCs) show greater proliferative potential than bone marrow stem cells, and their easier accessibility and limitless supply via liposuction of subcutaneous adipose tissue make them an ideal candidate for widespread therapeutic use. Jensen et al. demonstrated that human adipose-derived stromal cell treatment improved 7-day survival and mesenteric perfusion and attenuated the mucosal damage associated with intestinal I/R injury in rats [89]. They also discovered that human umbilical cord-derived mesenchymal stem cells (MSC) and bone marrow-derived MSCs yielded the same effect on improving survival rate of intestinal I/R rats [90]. Chang et al. reported that combined melatonin and adipose-derived mesenchymal stem cell treatment conferred additive beneficial effect against intestinal IR injury and was superior to either alone [91].

3.4 Conclusion

With the development of surgical operations, how to effectively protect the organs during the perioperative period has become a major field of surgery studies, and it is of great significance to improve the success rate of surgical treatment. But at present our clinical study on perioperative intestinal injury is still facing some difficulties, such as (1) an ideal clinical model is still lacking; (2) there is no uniform bowel dysfunction evaluation standard; and (3) the symptoms of intestinal injury itself are often concealed by that of parenteral organ dysfunction caused by intestinal injury. Therefore, the clinical study of perioperative intestinal injury will be a difficult and long-term process. We anesthesiologists must recognize the harmful consequences of perioperative intestinal injury and do our utmost to improve patients' eventual outcomes.

References

1. Zhao L, Luo L, Chen J, Xiao J, Jia W, Xiao Y. Utilization of extracorporeal membrane oxygenation alleviates intestinal ischemia-reperfusion injury in prolonged hemorrhagic shock animal model. Cell Biochem Biophys. 2014;70(3):1733–40. https://doi.org/10.1007/s12013-014-0121-3.
2. Slone EA, Fleming SD. Membrane lipid interactions in intestinal ischemia/reperfusion-induced injury. Clin Immunol. 2014;153(1):228–40. https://doi.org/10.1016/j.clim.2014.04.018.
3. Oldenburg W, Lau L, Rodenberg T, Edmonds H, Burger C. Acute mesenteric ischemia: a clinical review. Arch Intern Med. 2004;164(10):1054–62.
4. Smit M, Buddingh KT, Bosma B, Nieuwenhuijs VB, Hofker HS, Zijlstra JG. Abdominal compartment syndrome and intra-abdominal ischemia in patients with severe acute pancreatitis. World J Surg. 2016;40(6):1454–61. https://doi.org/10.1007/s00268-015-3388-7.
5. Tian R, Tan JT, Wang RL, Xie H, Qian YB, Yu KL. The role of intestinal mucosa oxidative stress in gut barrier dysfunction of severe acute pancreatitis. Eur Rev Med Pharmacol Sci. 2013;17(3):349–55.
6. Yang R, Tenhunen J, Tonnessen TI. HMGB1 and histones play a significant role in inducing systemic inflammation and multiple organ dysfunctions in severe acute pancreatitis. Int J Inflamm. 2017;2017:1817564. https://doi.org/10.1155/2017/1817564.
7. De Silva RJ, Bhinda P, Goddard M, Choong CK. The value of post mortems in cardiac surgery: learning from the dead. Heart Lung Circ. 2012;21(3):150–3. https://doi.org/10.1016/j.hlc.2011.11.005.
8. Adamik B, Kubler A, Gozdzik A, Gozdzik W. Prolonged cardiopulmonary bypass is a risk factor for intestinal ischaemic damage and endotoxaemia. Heart Lung Circ. 2017;26(7):717–23. https://doi.org/10.1016/j.hlc.2016.10.012.
9. Ultee KH, Zettervall SL, Soden PA, Darling J, Bertges DJ, Verhagen HJ, Schermerhorn ML. Incidence of and risk factors for bowel ischemia after abdominal aortic aneurysm repair. J Vasc Surg. 2016;64(5):1384–91. https://doi.org/10.1016/j.jvs.2016.05.045.
10. Li BC, Xia ZQ, Li C, Liu WF, Wen SH, Liu KX. The incidence and risk factors of gastrointestinal complications after hepatectomy: a retrospective observational study of 1329 consecutive patients in a single center. J Surg Res. 2014;192(2):440–6. https://doi.org/10.1016/j.jss.2014.06.015.
11. Nastos C, Kalimeris K, Papoutsidakis N, Tasoulis MK, Lykoudis PM, Theodoraki K, et al. Global consequences of liver ischemia/reperfusion injury. Oxidative Med Cell Longev. 2014;2014:906965. https://doi.org/10.1155/2014/906965.

12. Lenaerts K, Ceulemans LJ, Hundscheid IH, Grootjans J, Dejong CH, Olde Damink SW. New insights in intestinal ischemia-reperfusion injury: implications for intestinal transplantation. Curr Opin Organ Transplant. 2013;18(3):298–303. https://doi.org/10.1097/MOT.0b013e32835ef1eb.
13. Zhang HY, Wang F, Feng JX. Intestinal microcirculatory dysfunction and neonatal necrotizing enterocolitis. Chin Med J. 2013;126(9):1771–8.
14. Neu J. Necrotizing enterocolitis. World Rev Nutr Diet. 2014;110:253–63. https://doi.org/10.1159/000358474.
15. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014;94(2):329–54. https://doi.org/10.1152/physrev.00040.2012.
16. Mallick IH, Yang W, Winslet MC, Seifalian AM. Ischemia-reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci. 2004;49(9):1359–77.
17. Wen S, Ling Y, Yang W, Shen J, Li C, Deng W, et al. Necroptosis is a key mediator of enterocytes loss in intestinal ischaemia/reperfusion injury. J Cell Mol Med. 2017;21(3):432–43. https://doi.org/10.1111/jcmm.12987.
18. Wen SH, Li Y, Li C, Xia ZQ, Liu WF, Zhang XY, et al. Ischemic postconditioning during reperfusion attenuates intestinal injury and mucosal cell apoptosis by inhibiting JAK/STAT signaling activation. Shock. 2012;38(4):411–9. https://doi.org/10.1097/SHK.0b013e3182662266.
19. Li Y, Wen S, Yao X, Liu W, Shen J, Deng W, et al. MicroRNA-378 protects against intestinal ischemia/reperfusion injury via a mechanism involving the inhibition of intestinal mucosal cell apoptosis. Cell Death Dis. 2017;8(10):e3127. https://doi.org/10.1038/cddis.2017.508.
20. Chi X, Yao W, Xia H, Jin Y, Li X, Cai J, Hei Z. Elevation of HO-1 expression mitigates intestinal ischemia-reperfusion injury and restores tight junction function in a rat liver transplantation model. Oxidative Med Cell Longev. 2015;2015:986075. https://doi.org/10.1155/2015/986075.
21. Jin Y, Blikslager AT. Myosin light chain kinase mediates intestinal barrier dysfunction via occludin endocytosis during anoxia/reoxygenation injury. Am J Physiol Cell Physiol. 2016;311(6):C996–c1004. https://doi.org/10.1152/ajpcell.00113.2016.
22. Wells JM, Brummer RJ, Derrien M, MacDonald TT, Troost F, Cani PD, et al. Homeostasis of the gut barrier and potential biomarkers. Am J Physiol Gastrointest Liver Physiol. 2017;312(3):G171–g193. https://doi.org/10.1152/ajpgi.00048.2015.
23. Takizawa Y, Kishimoto H, Kitazato T, Tomita M, Hayashi M. Changes in protein and mRNA expression levels of claudin family after mucosal lesion by intestinal ischemia/reperfusion. Int J Pharm. 2012;426(1–2):82–9. https://doi.org/10.1016/j.ijpharm.2012.01.023.
24. Takizawa Y, Kishimoto H, Tomita M, Hayashi M. Changes in the expression levels of tight junction components during reconstruction of tight junction from mucosal lesion by intestinal ischemia/reperfusion. Eur J Drug Metab Pharmacokinet. 2014;39(3):211–20. https://doi.org/10.1007/s13318-013-0151-z.
25. Zhang XY, Liu ZM, Zhang HF, Li YS, Wen SH, Shen JT, Liu KX. Decreased PD-1/PD-L1 expression Is associated with the reduction in mucosal immunoglobulin A in mice with intestinal ischemia reperfusion. Dig Dis Sci. 2015;60(9):2662–9. https://doi.org/10.1007/s10620-015-3684-y.
26. Zhang XY, Liu ZM, Zhang HF, Li YS, Wen SH, Shen JT, et al. TGF-beta1 improves mucosal IgA dysfunction and dysbiosis following intestinal ischaemia-reperfusion in mice. J Cell Mol Med. 2016;20(6):1014–23. https://doi.org/10.1111/jcmm.12789.
27. Diebel LN, Liberati DM, Dulchavsky SA, Diglio CA, Brown WJ. Enterocyte apoptosis and barrier function are modulated by SIgA after exposure to bacteria and hypoxia/reoxygenation. Surgery. 2003;134(4):574–80. https://doi.org/10.1016/s0039. discussion 580-571.
28. Lee H, Green DJ, Lai L, Hou YJ, Jensenius JC, Liu D, et al. Early complement factors in the local tissue immunocomplex generated during intestinal ischemia/reperfusion injury. Mol Immunol. 2010;47(5):972–81. https://doi.org/10.1016/j.molimm.2009.11.022.
29. Shi T, Moulton VR, Lapchak PH, Deng GM, Dalle Lucca JJ, Tsokos GC. Ischemia-mediated aggregation of the actin cytoskeleton is one of the major initial events resulting in ischemia-

reperfusion injury. Am J Physiol Gastrointest Liver Physiol. 2009;296(2):G339–47. https://doi.org/10.1152/ajpgi.90607.2008.
30. Zhang M, Alicot E, Carroll M. Human natural IgM can induce ischemia/reperfusion injury in a murine intestinal model. Mol Immunol. 2008;45(15):4036–9.
31. Hu R, Chen ZF, Yan J, Li QF, Huang Y, Xu H, et al. Complement C5a exacerbates acute lung injury induced through autophagy-mediated alveolar macrophage apoptosis. Cell Death Dis. 2014;5:e1330. https://doi.org/10.1038/cddis.2014.274.
32. Tuboly E, Futakuchi M, Varga G, Erces D, Tokes T, Meszaros A, et al. C5a inhibitor protects against ischemia/reperfusion injury in rat small intestine. Microbiol Immunol. 2016;60(1):35–46. https://doi.org/10.1111/1348-0421.12338.
33. Chen J, Crispin JC, Tedder TF, Dalle Lucca J, Tsokos GC. B cells contribute to ischemia/reperfusion-mediated tissue injury. J Autoimmun. 2009;32(3–4):195–200. https://doi.org/10.1016/j.jaut.2009.02.021.
34. Grootjans J, Hodin CM, de Haan JJ, Derikx JP, Rouschop KM, Verheyen FK, et al. Level of activation of the unfolded protein response correlates with Paneth cell apoptosis in human small intestine exposed to ischemia/reperfusion. Gastroenterology. 2011;140(2):529–539. e523. https://doi.org/10.1053/j.gastro.2010.10.040.
35. Fukatsu K, Sakamoto S, Hara E, Ueno C, Maeshima Y, Matsumoto I, et al. Gut ischemia-reperfusion affects gut mucosal immunity: a possible mechanism for infectious complications after severe surgical insults. Crit Care Med. 2006;34(1):182–7.
36. Watson MJ, Ke B, Shen XD, Gao F, Busuttil RW, Kupiec-Weglinski JW, Farmer DG. Treatment with antithymocyte globulin ameliorates intestinal ischemia and reperfusion injury in mice. Surgery. 2012;152(5):843–50. https://doi.org/10.1016/j.surg.2012.03.001.
37. Yang X, Bai H, Wang Y, Li J, Zhou Q, Cai W, et al. Deletion of regulatory T cells supports the development of intestinal ischemia-reperfusion injuries. J Surg Res. 2013;184(2):832–7. https://doi.org/10.1016/j.jss.2013.05.014.
38. Karhausen J, Qing M, Gibson A, Moeser AJ, Griefingholt H, Hale LP, et al. Intestinal mast cells mediate gut injury and systemic inflammation in a rat model of deep hypothermic circulatory arrest. Crit Care Med. 2013;41(9):e200–10. https://doi.org/10.1097/CCM.0b013e31827cac7a.
39. Chen Y, Lui VC, Rooijen NV, Tam PK. Depletion of intestinal resident macrophages prevents ischaemia reperfusion injury in gut. Gut. 2004;53(12):1772–80. https://doi.org/10.1136/gut.2003.034868.
40. Liu WF, Wen SH, Zhan JH, Li YS, Shen JT, Yang WJ, et al. Treatment with recombinant trichinella spiralis cathepsin B-like protein ameliorates intestinal ischemia/reperfusion injury in mice by promoting a switch from M1 to M2 macrophages. J Immunol. 2015;195(1):317–28. https://doi.org/10.4049/jimmunol.1401864.
41. Towfigh S, Heisler T, Rigberg DA, Hines OJ, Chu J, McFadden DW, Chandler C. Intestinal ischemia and the gut-liver axis: an in vitro model. J Surg Res. 2000;88(2):160–4. https://doi.org/10.1006/jsre.1999.5767.
42. Salerno-Goncalves R, Safavie F, Fasano A, Sztein MB. Free and complexed-secretory immunoglobulin A triggers distinct intestinal epithelial cell responses. Clin Exp Immunol. 2016;185(3):338–47. https://doi.org/10.1111/cei.12801.
43. Hoehn RS, Seitz AP, Jernigan PL, Gulbins E, Edwards MJ. Ischemia/reperfusion injury alters sphingolipid metabolism in the gut. Cell Physiol Biochem. 2016;39(4):1262–70. https://doi.org/10.1159/000447831.
44. Liu KX, Chen SQ, Huang WQ, Li YS, Irwin MG, Xia Z. Propofol pretreatment reduces ceramide production and attenuates intestinal mucosal apoptosis induced by intestinal ischemia/reperfusion in rats. Anesth Analg. 2008;107(6):1884–91. https://doi.org/10.1213/ane.0b013e3181884bbf.
45. Liu KX, He W, Rinne T, Liu Y, Zhao MQ, Wu WK. The effect of ginkgo biloba extract (EGb 761) pretreatment on intestinal epithelial apoptosis induced by intestinal ischemia/reperfusion in rats: role of ceramide. Am J Chin Med. 2007;35(5):805–19. https://doi.org/10.1142/s0192415x07005284.

46. Wang F, Li Q, He Q, Geng Y, Tang C, Wang C, Li J. Temporal variations of the ileal microbiota in intestinal ischemia and reperfusion. Shock. 2013;39(1):96–103. https://doi.org/10.1097/SHK.0b013e318279265f.
47. Ozkan O, Ozkan O, Bektas G, Cinpolat A, Bassorgun I, Ciftcioglu A. The relationship between ischemia time and mucous secretion in vaginal reconstruction with the jejunal free flap: an experimental study on the rat jejunum. Ann Plast Surg. 2015;75(1):98–101. https://doi.org/10.1097/01.SAP.0000466781.69925.b2.
48. Castaneda A, Vilela R, Chang L, Mercer DW. Effects of intestinal ischemia/reperfusion injury on gastric acid secretion. J Surg Res. 2000;90(1):88–93. https://doi.org/10.1006/jsre.2000.5853.
49. Liu Z, Luo Y, Cheng Y, Zou D, Zeng A, Yang C, et al. Gastrin attenuates ischemia-reperfusion-induced intestinal injury in rats. Exp Biol Med (Maywood). 2016;241(8):873–81. https://doi.org/10.1177/1535370216630179.
50. Chang M, Alsaigh T, Kistler EB, Schmid-Schonbein GW. Breakdown of mucin as barrier to digestive enzymes in the ischemic rat small intestine. PLoS One. 2012;7(6):e40087. https://doi.org/10.1371/journal.pone.0040087.
51. Fishman JE, Sheth SU, Levy G, Alli V, Lu Q, Xu D, et al. Intraluminal nonbacterial intestinal components control gut and lung injury after trauma hemorrhagic shock. Ann Surg. 2014;260(6):1112–20. https://doi.org/10.1097/SLA.0000000000000631.
52. Qin X, Sheth SU, Sharpe SM, Dong W, Lu Q, Xu D, Deitch EA. The mucus layer is critical in protecting against ischemia-reperfusion-mediated gut injury and in the restitution of gut barrier function. Shock. 2011;35(3):275–81. https://doi.org/10.1097/SHK.0b013e3181f6aaf1.
53. Grootjans J, Hundscheid IH, Lenaerts K, Boonen B, Renes IB, Verheyen FK, et al. Ischaemia-induced mucus barrier loss and bacterial penetration are rapidly counteracted by increased goblet cell secretory activity in human and rat colon. Gut. 2013;62(2):250–8. https://doi.org/10.1136/gutjnl-2011-301956.
54. Zhou J, Huang WQ, Li C, Wu GY, Li YS, Wen SH, et al. Intestinal ischemia/reperfusion enhances microglial activation and induces cerebral injury and memory dysfunction in rats. Crit Care Med. 2012;40(8):2438–48. https://doi.org/10.1097/CCM.0b013e3182546855.
55. Liu KX, Chen SQ, Zhang H, Guo JY, Li YS, Huang WQ. Intestinal ischaemia/reperfusion upregulates beta-defensin-2 expression and causes acute lung injury in the rat. Injury. 2009;40(9):950–5. https://doi.org/10.1016/j.injury.2009.01.103.
56. Lin ZL, Tan SJ, Cheng MH, Zhao CY, Yu WK, He YL, et al. Lipid-rich enteral nutrition controls intestinal inflammation, improves intestinal motility and mucosal barrier damage in a rat model of intestinal ischemia/reperfusion injury. J Surg Res. 2017;213:75–83. https://doi.org/10.1016/j.jss.2017.02.007.
57. Wu C, Wang X, Jiang T, Li C, Zhang L, Gao X, et al. Partial enteral nutrition mitigated ischemia/reperfusion-induced damage of rat small intestinal barrier. Nutrients. 2016;8(8):502. https://doi.org/10.3390/nu8080502.
58. Shu X, Zhang J, Wang Q, Xu Z, Yu T. Glutamine decreases intestinal mucosal injury in a rat model of intestinal ischemia-reperfusion by downregulating HMGB1 and inflammatory cytokine expression. Exp Ther Med. 2016;12(3):1367–72. https://doi.org/10.3892/etm.2016.3468.
59. Peng Z, Ban K, Wawrose RA, Gover AG, Kozar RA. Protection by enteral glutamine is mediated by intestinal epithelial cell peroxisome proliferator-activated receptor-gamma during intestinal ischemia/reperfusion. Shock. 2015;43(4):327–33. https://doi.org/10.1097/shk.0000000000000297.
60. Kim Y, Kim DC, Cho ES, Ko SO, Kwon WY, Suh GJ, Shin HK. Antioxidant and anti-inflammatory effects of selenium in oral buccal mucosa and small intestinal mucosa during intestinal ischemia-reperfusion injury. J Inflamm (Lond). 2014;11(1):36. https://doi.org/10.1186/s12950-014-0036-1.
61. Colak T, Ozturk C, Polat A, Bagdatoglu O, Kanik A, Turkmenoglu O, Aydin S. Effects of trapidil on intestinal mucosal barrier function and bacterial translocation after intestinal ischemia and reperfusion in an experimental rat model. Curr Ther Res Clin Exp. 2003;64(6):355–66. https://doi.org/10.1016/s0011-393x(03)00091-2.

62. Colak T, Polat A, Bagdatoglu O, Kanik A, Turkmenoglu O, Aydin S. Effect of trapidil in ischemia/reperfusion injury on rat small intestine. J Investig Surg. 2003;16(3):167–76.
63. Ben Shahar Y, Sukhotnik I, Bitterman N, Pollak Y, Bejar J, Chepurov D, et al. Effect of N-acetylserotonin on intestinal recovery following intestinal ischemia-reperfusion injury in a rat. Eur J Pediatr Surg. 2016;26(1):47–53. https://doi.org/10.1055/s-0035-1559886.
64. Tas U, Ayan M, Sogut E, Kuloglu T, Uysal M, Tanriverdi HI, et al. Protective effects of thymoquinone and melatonin on intestinal ischemia-reperfusion injury. Saudi J Gastroenterol. 2015;21(5):284–9. https://doi.org/10.4103/1319-3767.166203.
65. Yang B, Ni YF, Wang WC, Du HY, Zhang H, Zhang L, et al. Melatonin attenuates intestinal ischemia--reperfusion-induced lung injury in rats by upregulating N-myc downstream-regulated gene 2. J Surg Res. 2015;194(1):273–80. https://doi.org/10.1016/j.jss.2014.11.018.
66. Jiang LL, Zhang JJ, Zhang ZZ, He XH, Chen DL, Wang YL. Effect of intraperitoneal resuscitation with different concentrations of sodium pyruvate on intestinal ischemia reperfusion injury in hemorrhagic shock rat. Shock. 2016;45(4):441–9. https://doi.org/10.1097/shk.0000000000000515.
67. Zhang JJ, Zhang ZZ, Ke JJ, He XH, Zhan J, Chen DL, et al. Protection against intestinal injury from hemorrhagic shock by direct peritoneal resuscitation with pyruvate in rats. Shock. 2014;42(5):464–71. https://doi.org/10.1097/shk.0000000000000230.
68. Tassopoulos A, Chalkias A, Papalois A, Iacovidou N, Xanthos T. The effect of antioxidant supplementation on bacterial translocation after intestinal ischemia and reperfusion. Redox Rep. 2017;22(1):1–9. https://doi.org/10.1080/13510002.2016.1229893.
69. Borges SC, da Silva De Souza AC, Beraldi EJ, Schneider LC, Buttow NC. Resveratrol promotes myenteric neuroprotection in the ileum of rats after ischemia-reperfusion injury. Life Sci. 2016;166:54–9. https://doi.org/10.1016/j.lfs.2016.09.016.
70. Huang X, Zhao W, Hu D, Han X, Wang H, Yang J, et al. Resveratrol efficiently improves pulmonary function via stabilizing mast cells in a rat intestinal injury model. Life Sci. 2017;185:30–7. https://doi.org/10.1016/j.lfs.2017.07.018.
71. Xu X, Li D, Gao H, Gao Y, Zhang L, Du Y, et al. Protective effect of the traditional Chinese medicine xuesaitong on intestinal ischemia-reperfusion injury in rats. Int J Clin Exp Med. 2015;8(2):1768–79.
72. Jiang Y, Zhou Z, Meng QT, Sun Q, Su W, Lei S, et al. Ginsenoside Rb1 treatment attenuates pulmonary inflammatory cytokine release and tissue injury following intestinal ischemia reperfusion injury in mice. Oxidative Med Cell Longev. 2015;2015:843721. https://doi.org/10.1155/2015/843721.
73. Zhu J, Wang P, He Q, Zhou J, Luo C. Evidence of an anti-apoptotic effect of qinghuobaiduyin on intestinal mucosa following burn injury. Exp Ther Med. 2013;6(6):1390–6. https://doi.org/10.3892/etm.2013.1314.
74. Mo LQ, Chen Y, Song L, Wu GM, Tang N, Zhang YY, et al. Osthole prevents intestinal ischemia-reperfusion-induced lung injury in a rodent model. J Surg Res. 2014;189(2):285–94. https://doi.org/10.1016/j.jss.2014.03.026.
75. Shen JT, Li YS, Xia ZQ, Wen SH, Yao X, Yang WJ, et al. Remifentanil preconditioning protects the small intestine against ischemia/reperfusion injury via intestinal delta- and mu-opioid receptors. Surgery. 2016;159(2):548–59. https://doi.org/10.1016/j.surg.2015.07.028.
76. Zhang XY, Liu ZM, Wen SH, Li YS, Li Y, Yao X, et al. Dexmedetomidine administration before, but not after, ischemia attenuates intestinal injury induced by intestinal ischemia-reperfusion in rats. Anesthesiology. 2012;116(5):1035–46. https://doi.org/10.1097/ALN.0b013e3182503964.
77. Gan X, Xing D, Su G, Li S, Luo C, Irwin MG, et al. Propofol attenuates small intestinal ischemia reperfusion injury through inhibiting NADPH oxidase mediated mast cell activation. Oxidative Med Cell Longev. 2015;2015:167014. https://doi.org/10.1155/2015/167014.
78. Zhao W, Zhou S, Yao W, Gan X, Su G, Yuan D, Hei Z. Propofol prevents lung injury after intestinal ischemia-reperfusion by inhibiting the interaction between mast cell activation and oxidative stress. Life Sci. 2014;108(2):80–7. https://doi.org/10.1016/j.lfs.2014.05.009.

79. Gan X, Su G, Zhao W, Huang P, Luo G, Hei Z. The mechanism of sevoflurane preconditioning-induced protections against small intestinal ischemia reperfusion injury is independent of mast cell in rats. Mediat Inflamm. 2013;2013:378703. https://doi.org/10.1155/2013/378703.
80. Liu C, Shen Z, Liu Y, Peng J, Miao L, Zeng W, Li Y. Sevoflurane protects against intestinal ischemia-reperfusion injury partly by phosphatidylinositol 3 kinases/Akt pathway in rats. Surgery. 2015;157(5):924–33. https://doi.org/10.1016/j.surg.2014.12.013.
81. Kim M, Park SW, Kim M, D'Agati VD, Lee HT. Isoflurane post-conditioning protects against intestinal ischemia-reperfusion injury and multiorgan dysfunction via transforming growth factor-beta1 generation. Ann Surg. 2012;255(3):492–503. https://doi.org/10.1097/SLA.0b013e3182441767.
82. Harm S, Gabor F, Hartmann J. Low-dose polymyxin: an option for therapy of Gram-negative sepsis. Innate Immun. 2016;22(4):274–83. https://doi.org/10.1177/1753425916639120.
83. Ji YY, Wang ZD, Wang SF, Wang BT, Yang ZA, Zhou XR, et al. Ischemic preconditioning ameliorates intestinal injury induced by ischemia-reperfusion in rats. World J Gastroenterol. 2015;21(26):8081–8. https://doi.org/10.3748/wjg.v21.i26.8081.
84. Liu KX, Li YS, Huang WQ, Chen SQ, Wang ZX, Liu JX, Xia Z. Immediate postconditioning during reperfusion attenuates intestinal injury. Intensive Care Med. 2009;35(5):933–42. https://doi.org/10.1007/s00134-009-1428-1.
85. Wen SH, Ling YH, Li Y, Li C, Liu JX, Li YS, et al. Ischemic postconditioning during reperfusion attenuates oxidative stress and intestinal mucosal apoptosis induced by intestinal ischemia/reperfusion via aldose reductase. Surgery. 2013;153(4):555–64. https://doi.org/10.1016/j.surg.2012.09.017.
86. Rosero O, Onody P, Kovacs T, Molnar D, Fulop A, Lotz G, et al. Postconditioning: "Tollerating" mesenteric ischemia-reperfusion injury? Surgery. 2017;161(4):1004–15. https://doi.org/10.1016/j.surg.2016.09.031.
87. Li C, Li YS, Xu M, Wen SH, Yao X, Wu Y, et al. Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial. Anesthesiology. 2013;118(4):842–52. https://doi.org/10.1097/ALN.0b013e3182850da5.
88. Shen ZY, Zhang J, Song HL, Zheng WP. Bone-marrow mesenchymal stem cells reduce rat intestinal ischemia-reperfusion injury, ZO-1 downregulation and tight junction disruption via a TNF-alpha-regulated mechanism. World J Gastroenterol. 2013;19(23):3583–95. https://doi.org/10.3748/wjg.v19.i23.3583.
89. Jensen AR, Doster DL, Hunsberger EB, Manning MM, Stokes SM, Barwinska D, et al. Human adipose stromal cells increase survival and mesenteric perfusion following intestinal ischemia and reperfusion injury. Shock. 2016;46(1):75–82. https://doi.org/10.1097/shk.0000000000000571.
90. Jensen AR, Manning MM, Khaneki S, Drucker NA, Markel TA. Harvest tissue source does not alter the protective power of stromal cell therapy after intestinal ischemia and reperfusion injury. J Surg Res. 2016;204(2):361–70. https://doi.org/10.1016/j.jss.2016.05.006.
91. Chang CL, Sung PH, Sun CK, Chen CH, Chiang HJ, Huang TH, et al. Protective effect of melatonin-supported adipose-derived mesenchymal stem cells against small bowel ischemia-reperfusion injury in rat. J Pineal Res. 2015;59(2):206–20. https://doi.org/10.1111/jpi.12251.

4 The Role of Mitochondrial Quality Imbalance in Multiple Organ Dysfunction Syndrome Following Severe Trauma, Shock, and Sepsis

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Abstract

Multiple organ dysfunction syndrome (MODS) is a life-threatening condition with high morbidity and mortality. Mitochondria are multifunctional organelles, whose failure triggers multiple organ dysfunction and is directly associated with patient's vicious outcome. Physiologically, mitochondria undergo continuous fission, fusion, biogenesis, and mitophagy (selective mitochondrial autophagy) to maintain homeostasis, whose disruption may heavily impact the mitochondrial quality and result in damaged cell and organ functions under pathological conditions such as severe trauma, shock, and sepsis. Mitochondrial quality imbalance is a key step in MODS process, and rebalancing the mitochondrial quality may be a promising approach in the treatment of MODS following severe trauma, shock, and sepsis.

Keywords

MODS · Mitochondrial quality · Intensive care medicine · Severe trauma · Mitochondrial dynamics · Mitophagy

4.1 Introduction

As medical techniques greatly develop, some diseases have been effectively controlled or got better care; however, severe trauma, shock, sepsis, severe pancreatitis, etc. which induced multiple organ dysfunction syndrome (MODS) have not been controlled well yet. It is reported that approximately 50% of patients in ICU can develop MODS, and their mortality is up to 30–100%. Patients with two organ

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dysfunction have more than 70% mortality rate [1]. However, such high mortality has not changed much since the 1980s.

Several mechanisms have been put forward to explain the process of MODS, such as immunosuppression and hypometabolism, ischemia and hypoxia induced by hypoperfusion, uncontrolled inflammation-induced extensive tissue damage, and so on. Some measures have been widely applied, such as anti-infection therapy, blood purification and immune adsorption, microcirculation modulation and hemodynamic support, etc., which made some contributions to the improvement of the outcome to these critical diseases. However, studies toward further understanding of MODS are still necessary. This review will focus on the mitochondrial quality imbalance and its role in MODS following severe trauma, shock, and sepsis; this might help to provide the better prevention and management of MODS.

4.2 Role of Mitochondrial Quality Imbalance in Diseases

Mitochondria are power houses of cells and responsible for aerobic respiration. Mitochondria not only produce ATP through oxidative phosphorylation but also participate in the regulation of metabolism, calcium homeostasis [2], oxidative signaling [3], steroid synthesis [4], etc. In pathologic conditions, mitochondria also induce oxidative injury and calcium overload, initiate cell apoptosis, etc. Mitochondria undergo continuous fission, fusion, biogenesis, and mitophagy to maintain the good quality and function of mitochondrial population. More and more studies revealed that mitochondrial quality imbalance plays important role in various pathological conditions.

4.2.1 Role of Mitochondria Dysfunction in Various Diseases

Mitochondrial dysfunction is involved in a wide range of clinical diseases, ranging from heart and brain diseases and diabetes to acute illnesses such as sepsis, traumatic injuries, and poisoning. As mitochondria are the center of cellular function and energy production, it is not surprising that these disease processes result in bioenergetic dysfunction [5]. Oxidative injury is considered as another major source of cell damage in various organ systems. Oxidative stress initiates diverse pathological shifts, such as mitochondrial DNA (mtDNA) mutation and apoptosis [6], and eventually boosts cell injuries. Since ROS generates in mitochondria, mitochondria are easily attacked by ROS. ROS-induced mitochondrial DNA (mtDNA) mutation and depletion would result in more downstream mitochondrial stresses [6].

Oxidative stress is one of the important pathological factors of chronic diseases including cardiovascular diseases (i.e., atherosclerosis, hypertension, cardiomyopathy, and congestive heart failure), neurodegeneration diseases (i.e., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis), asthma and chronic obstructive pulmonary disease (COPD), and renal failure. In acute pathologies such

as trauma, stroke, and acute myocardial infarction, oxidative stress is also an important inducing factor of secondary injury of organs and tissues.

Besides, mitochondria store calcium and sustain calcium hemostasis, which are the important cytosolic buffer for calcium. In pathological conditions, mitochondrial permeability transition pore (mPTP) and mitochondrial calcium uniporter (MCU) complex might mediate excessive calcium inflow toward mitochondria and provoke calcium overload, which induces apoptosis afterward [7–9]. Intensive long-lasting excitatory stimuli eventually cause a persistent mitochondrial calcium accumulation and cytotoxicity; this is a mechanism that has been implicated in the pathogenesis of neurological disorders such as Alzheimer's and Huntington's disease and schizophrenia [10]. Mitochondria-derived cell injury can finally initiate intrinsic apoptosis, which has been widely studied in many cell types (as reviewed by Wu et al.) [11], including immune cells, cardiomyocytes, hepatocytes, hemotocytes, etc. Damaged mitochondria can be fixed or cleared to avoid the final detrimental effect. This process relies on mitochondrial quality control. However, the underlying mechanism for mitochondrial quality control is not fully understood yet.

4.2.2 Mitochondrial Quality Balance Is a Dynamic Process to Regulate Mitochondrial Function

Continuous fission, fusion, mitophagy, and biogenesis sustain mitochondrial quality balance, including adequate mitochondrial mass and function, which is indispensable for normal cell function. In mitochondria fission, parental mitochondrion divides into at least two mitochondria to achieve cell mitogen or meet energy demand; in mitochondria fusion, mitochondria merge to achieve self-renewal and promote mitochondrial function; in mitophagy, damaged mitochondria are segregated and degraded by selective autophagy process to diminish detrimental effects; in mitochondria biogenesis, mitochondrial functional or structural proteins are synthesized to replenish mitochondrial pool.

Basic studies indicated that mitochondrial characteristics vary from one kind of cell to another. Mitochondria account for ~40% of cardiomyocytes cell volume while 10–20% in vascular smooth muscle cells or vascular endothelia cells; cardiomyocyte completes rejuvenation in 2–3 days while vascular endothelial cells or intestinal epithelial cells in 2–3 h. Despite such heterogeneity, mitochondria share almost common fission, fusion, mitophagy, and biogenesis mechanisms (Fig. 4.1).

4.2.2.1 Mitochondrial Fission

Mitochondrial fission is essential for stabilizing mitochondrial genome, regulating energy production, and modulating oxidative signaling [12–15]. The mechanism of mitochondrial fission is complex and has attracted much attention recently. Dynamin-related protein (DRP1) is recruited from cytoplasm to bind receptors (e.g., FIS1, MFF, Mid49/51) on mitochondria outer membrane [16]. Then, DRP1 oligomerizes and hydrolizes GTP to mediate constriction of the fission site [17, 18]. DRP1 possesses an N-terminal GTPase domain thought to provide mechanical

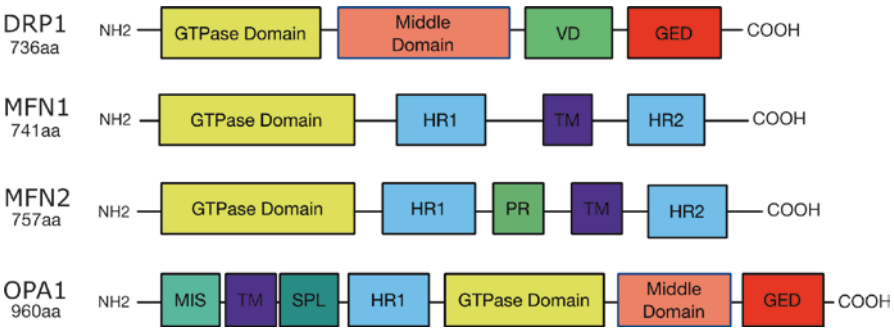

Fig. 4.1 Schematics of functional domains of the mitochondrial dynamic proteins. *HR* heptad repeat domain, *TM* transmembrane domain, *PR* proline-rich domain, *MIS* mitochondrial import sequence, *GED* GTPase effector domain, *VD* variable domain

force, a dynamin-like middle domain, and a GTPase effector domain (GED) located in the C-terminal region domain. DRP1 is predicted to exist as a T-shaped dimer or tetramer that contains a head (containing GTPase domain), leg (VD), and stalk (middle and GED domains). GTP induces the rearrangement of the head and stalk, which generates a force ultimately resulting in membrane constriction [19].

Phosphorylation, ubiquitination, S-nitrosylation, SUMOylation, and O-GlcNAcylation of DRP1 regulate mitochondrial fission. The phosphorylation of DRP1 is the most studied. Generally, the phosphorylation of DRP1 Ser616 activates DRP1, while DRP1 Ser637/600/716/656 phosphorylation inhibits it [19]. CaMKII [20], calcineurin [21, 22], PINK1 [23], Parkin [24], RhoA/ROCK pathway [25, 26], ERK [27, 28], cyclins [29], and Cdk5 [30] are proven to be responsible for the phosphorylation or dephosphorylation of DRP1. March5 (also known as MITOL) may ubiquitinate DRP1 or MiD49 on the MOM and reduces mitochondrial fragmentation [31–33]. S-nitrosylation of DRP1 at Cys-644 may enhance GTPase activity and DRP1 oligomerization and results in excessive mitochondrial fission in neurons and neuronal damage [34]. In addition, increased DRP1 SUMOylation by MAPL overexpression may upregulate mitochondrial fission [35], and decreased DRP1 SUMOylation (i.e., deSUMOylation) by SENP5 may rescue the mitochondrial fission [36]; O-GlcNAcylation at Thr-585 and Thr-586 may reduce the mitochondrial fragmentation [37].

4.2.2.2 Mitochondrial Fusion

The most important function of mitochondrial fusion is to facilitate the heterogeneous mitochondria to mix and exchange content. Mitochondrial fusion participates in the quality control of mtDNA [12] and energy metabolism regulation, e.g., ameliorating oxidative stress, cell differentiation, stress adaption and steroidogenesis, and so on [38–40].

Since mitochondria are double-membrane bound organelles, the complete fusion calls for merging of both the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM), either process possesses different mechanisms,

respectively. MFN1 and MFN2 form homo-oligomers or hetero-oligomers (i.e., MFN1/MFN1, MFN2/MFN2, or MFN1/MFN2) to mediate OMM fusion [41]. OPA1 is responsible for IMM fusion and cristae morphology formation [42, 43]. Mitofusin harbors a GTPase domain close to the N-terminus that is involved in the GTP hydrolysis. Two hydrophobic heptad repeat (HR) domains are localized in the middle (HR1) and C-terminal (HR2) regions and provide the basis for most coiled-coil interactions. The HR2 domain was shown to be of great importance for mitochondrial fusion activity; formation of HR2 dimers promotes the generation of a mitochondrial tether before mitochondrial fusion. OPA1 contains an N-terminal mitochondrial localization sequence, responsible for import of the protein into the mitochondrial inner membrane. This region also has three putative cleavage sites for the mitochondrial processing peptidase. Other structural motifs include a transmembrane domain that anchors OPA1 in the mitochondrial inner membrane; the first coiled-coil domain, involved in protein-protein interactions; a GTPase domain crucial for protein activity; the middle domain, which participates in tetramerization and higher-order assembly of OPA1; and a second coiled-coil domain in the C-terminus that mediates the interaction between OPA1 and MFN1/2 [44], but the exact mechanism in which OPA1 drives IMM fusion is far from being elucidated. Expression level of MFNs or OPA1 influences mitochondrial fusion. Mitochondrial fusion is also regulated by ubiquitination, deacetylation of MFNs, or protease-dependent cleavage of OPA1 by OMA1, YME1L. MFN1/MFN2 are generally regulated at transcriptional and post-transcriptional levels. Upregulation of MFN1/MFN2 may result in the increased fusion [40, 45, 46], and downregulation of MFN1 or MFN2 may result in the decreased fusion [47, 48]. Deacetylation of MFN1 by HDAC6 may increase the fusion in fasting mice skeletal cells so as to produce more energy [49]. Ubiquitination of MFNs by USP30 or Parkin may result in the reduced fusion, which contributes to selectively isolate damaged mitochondria [50, 51]. Parra et al. found OPA1 upregulation induced by insulin in cardiomyocytes resulted in the increased mitochondrial fusion, along with the increase of the intracellular ATP levels and oxygen consumption [52]. Exercise pretreatment is protective in nervous system ischemia/reperfusion injury via upregulation of OPA1 and mitochondrial fusion [53]. Besides, protease-dependent cleavage seems to conduct the functional shift of OPA1. OPA1 could be processed into long form (L-OPA1) and short form (S-OPA1) possessing different functions, respectively. Mitochondrial fusion regulation is thought to depend on the coordination of L- and S-OPA1 [54]. Two key players for OPA1 proteolysis are the IMM peptidase OMA1 and the i-AAA protease YME1L. Loss function study of YME1L suggests that it is responsible for constitutive processing of OPA1 [54, 55], while OMA1 regulates stress-induced OPA1 cleavage [56]. But the role of OMA1 and YME1L in mitochondrial morphology remains controversial. Anand et al. found that long OPA1 forms were sufficient to mediate mitochondrial fusion in these cells and expression of short OPA1 forms promoted mitochondrial fragmentation, demonstrating a dispensable role of OPA1 processing in mitochondrial fusion and a stimulatory role of S-OPA1 [57]. Detailed studies into mitochondrial fusion mechanism are eagerly needed to facilitate the adequate manipulation of mitochondrial dynamics, which may further contribute to mitochondrial function management.

4.2.2.3 Mitophagy

Mitophagy is a selective autophagic process conserved in eukaryotes and plays an essential role in mitochondrial quality and quantity control. It is involved in myogenic differentiation, cardiomyocyte mitochondrial plasticity and metabolic transitioning of perinatal hearts [58], and metabolism regulation in the liver [59] and β cells [60]. Mitophagy can be classified into ubiquitin-dependent and receptor-dependent pathways. A ubiquitin/PINK1/Parkin-dependent mitophagy pathway was unraveled and was extensively characterized, in short: (1) damaged mitochondria are isolated by fission mechanisms and PINK1 is preserved on OMM; (2) ubiquitin chain is connected to OMM proteins (e.g., MFNs, DRP1, FIS1) of damaged mitochondria by the E3 ubiquitin ligase Parkin, which is activated by PINK1; (3) adaptors (p62/SQSTM1, optineurin/OPTN, NBR1, etc.) associate ubiquitin with membrane protein LC3 through their LC3-interacting region (LIR); and (4) damaged mitochondria are encapsulated by autophagosome and degraded in autophagolysosome. Besides, the consistent mitochondrial outer membrane receptors FUNDC1, NIX/BNIP3L, BNIP3, and Bcl2L13 mediate receptor-dependent mitophagy; they interact with LC3 directly through LIR and promote encapsulation and latter processes [61, 62].

PINK1 serves as the sensor for the mitochondrial polarization state. Mitochondrial depolarization inactivates its import and proteasomal degradation, leading to PINK1 accumulation on the OMM, then PINK1 phosphorylates MFN2 (at serine 442, threonine 111, etc.), and phosphorylated MFN2 can act as a receptor to recruit Parkin, an E3 ubiquitin ligase. PINK1 also phosphorylates ubiquitin at serine 65 and the ubiquitin-like domain of Parkin at serine 65, which recruit more Parkin to OMM and activate its E3 ligase activity. As for adaptors, p62 and NBR1 were found to be dispensable, and primary but redundant autophagy receptor functions were defined for OPTN and NDP52. USP8 deubiquitylation of auto-ubiquitylated Parkin is required for its localization to depolarized mitochondria and thereby for efficient activation of mitophagy. On the other hand, the ubiquitin-specific proteases USP30, which localizes at the OMM via a transmembrane domain, and USP15, which can fractionally localize to mitochondria, remove Parkin-ligated ubiquitin from OMM proteins and reduce mitophagy. In addition, mitophagy receptors upon expression, constitutively localize at the OMM via transmembrane domains, are transcriptionally regulated, and engagement of mitophagy receptor activity is controlled through the phosphorylation status of their LIR. Phosphorylation of serine residues 17 and 24 flanking the Bnip3 LIR specifically promotes binding to LC3B and GATE-16, but to date it remains undetermined which kinases and phosphatases are responsible for controlling the phosphorylation state of the Bnip3. In response to hypoxia or mitochondrial uncoupling, PGAM5 dephosphorylates CK2-phosphorylated serine 13 of FUNDC1 to activate LC3 binding. In addition, ULK1 phosphorylates serine 17 of the FUNDC1 LIR motif, resulting in increased LC3 binding [63].

4.2.2.4 Mitochondrial Biogenesis

Mitochondrial biogenesis includes synthesis of mtDNA-encoded protein, synthesis and import of nuclear-encoded proteins, assembly of the dual genetic origin-derived proteins and mtDNA replication, and finally formation of new organelle structures

[64]. Nuclear-encoded mitochondrial proteins are synthesized in cytoplasm and are then imported into mitochondria. mtDNA-encoded proteins are synthesized within the organelle itself.

The regulation of mitochondrial biogenesis includes a set of nuclear transcription factors. The nuclear transcription factor, NRF1, governs the expression of nuclear OXPHOS genes as well as the expression of nuclear-encoded factors involved in mitochondrial transcription, protein import, and protein assembly. NRF2 binding sites have been also identified in several other mitochondrial genes including the OXPHOS subunits, mitochondrial protein import machinery, and mitochondrial translation factors. Additional transcription factors such as the estrogen-related receptor α (ERRα), cAMP response element-binding protein (CREB), and Yin Yang 1 (YY1) are also involved. A higher level of regulation is achieved by the family of coactivators of the peroxisome proliferator-activated receptors (PPARs). The best studied member of this family is the PPAR coactivator 1α (PGC-1α), which is termed the master regulator of mitochondrial biogenesis. PGC-1α expression is upregulated by PPARs, CREB, and YY1/mTOR and is downregulated by RIP140, 160MYB, DNMT3b, p53, etc. Besides, PGC-1α can be phosphorylated by p38, MAPK, and AMPK and inhibited by Akt. Activation of Sirt1 and the deacetylation of PGC-1α can also activate PGC-1α [65].

Mitochondrial biogenesis and mitochondrial mass can be modulated through several stimuli and cellular pathways, including but not limited to hormones such as thyroid hormone [66] and estrogen [67], inflammatory signaling [68], as well as calcium signaling [69]. As a result, mitochondrial biogenesis plays vital role in cell differentiation [70–72], immune response [73–75], and inflammation response in the liver, kidney, heart, and lung [76–79].

4.2.3 Role of Mitochondrial Quality Imbalance in Various Diseases

Diversified stimuli disrupt such dynamics by hindering any of the above four processes and results in mitochondrial quality imbalance, which induces functional impairment or structural damage in mitochondria and strongly insults organ function [80].

Mitochondrial quality imbalance is largely seen in neurodegeneration [81, 82]. Downregulation of MFN2 may cause mitochondrial dysfunction, alter calcium homeostasis, and enhance Bax translocation to mitochondria, resulting in delayed neuronal death in in vitro and in vivo models of excitotoxicity [48]. Defect in mitophagy may result in damaged mitochondria accumulation and neurodegeneration [83]. Loss of PINK1 function is associated with early-onset recessive Parkinson's disease, in vitro studies showed that cells lacking Pink1 had lower DRP1 and MFN2 expression, and mitochondrial morphology was fragmented [84]. Impaired mitochondrial biogenesis contributed to mitochondrial dysfunction in Alzheimer's disease [85]. TNF-α may activate NF-κB signaling and increase OPA1 expression, while IL-6 may upregulate fission inducer FIS1 and downregulate

MFN2, both signal axes contributed to islet cell apoptosis and type 2 diabetes [86]. Disturbances in mitochondrial biogenesis and PGC-1α levels are involved in type 2 diabetes, neurodegenerative disease, and many age-related pathologies [87, 88].

Mitochondrial quality imbalance also participates in infection and inflammatory diseases. *S. mansoni* infection may change hepatocyte mitochondrial morphology and affect mitochondrial function, in which mitochondrial biogenesis and fission were also upregulated [89]. The toxic bile salt glycochenodeoxycholate-induced mitochondrial fragmentation was associated with an increase in ROS levels and hepatic cell death [90]. Induction of mitochondrial fission by cathepsin E in lung epithelial contributed to increased caspase activation/apoptosis, and lung epithelial-targeted transgenic cathepsin E mice developed emphysema similarly [91].

Mitochondrial quality balance is also studied in the cardiovascular system. Rat cardiac arrest model showed excessive autophagy and mitophagy, along with increased apoptosis in cardiomyocytes [92]. Genetic ablation of both MFN1 and MFN2 in the adult murine heart resulted in mitochondrial fragmentation, impairment in mitochondrial respiration, and a severe lethal cardiomyopathy after 7–8 weeks [93, 94]. Upregulation of miR-140 and downregulation of MFN1 were found in right ventricles of pulmonary arterial hypertension rats, which correlated with increased right ventricular systolic pressure and hypertrophy [95].

4.3 Role of Mitochondrial Quality Imbalance in MODS Following Severe Trauma, Shock, and Sepsis

4.3.1 Role of Mitochondrial Dysfunction in MODS Following Severe Trauma, Shock, and Sepsis

Studies revealed pivotal role of mitochondria dysfunction in MODS, and mitochondrial status is directly associated with patient outcome [96]. Decreased mitochondrial complex I activity was associated with the degree of nitric oxide (NO) production in the skeletal muscle of patients admitted to intensive care unit with septic shock [97]. Respiratory protein subunits and transcripts were depleted in critically ill patients [98]. In rat sepsis models induced by cecal ligation and puncture (CLP), Karlsson et al. found a mismatch between reduced oxygen delivery and increased oxygen demand which impaired mitochondria and vital organs including the brain and liver [99]. Besides, adenosine diphosphate (ADP)-stimulated respiratory rates of cardiac fibers were reduced in septic mice due to reduced Ser-58 phosphorylation of cytochrome c oxidase subunit IV-1, resulted in cardiac dysfunction [100]. LPS reduced ATP content in HepG2 cell and primary human hepatocytes, partly by modulating complex II respiration [101]. LPS significantly induced heart oxidative stress and abnormal oxidative phosphorylation, which further impair cardiac contractile and bioenergetic function [25]. In addition, Herminghaus A et al. investigated the varying degrees of sepsis on hepatic mitochondrial function and related varied respiratory control ratio (RCR) to the severity of sepsis [102]. Joseph L et al. found the increased cardiac oxidative stress and decreased systolic function

were accompanied with mitochondrial calcium overload and depolarization of the mitochondrial inner membrane potential in a mouse model of endotoxemia [103]. Besides, studies also showed increased renal tubular cell [104] and endothelial cell [105] apoptosis by LPS stimulus. In addition, in experimental models of hemorrhagic shock and resuscitation in murine and porcine, mitochondrial injury was observed, as well as cell injury and organ dysfunction [106]. These studies remind us of the fact that mitochondrial respiration is impaired and decreased mitochondria function is closely related to organ function in sepsis and other critical illness.

4.3.2 Possible Role of Mitochondrial Quality Imbalance in MODS Following Severe Trauma, Shock, and Sepsis

As above discussed, mitochondrial quality imbalance has been studied in chronic pathologies, such as neurodegeneration, diabetes, and inflammatory diseases. However, mitochondria are highly dynamic organelles, and acute stresses including trauma, shock- and sepsis-induced ischemia, hypoxia, endotoxemia, and cytokines could induce rapid changes in mitochondrial morphology and function in multiple organs (Fig. 4.2).

Dysregulated fission and fusion are widely reported. In a traumatic rat model, the number of heart mitochondria was increased, while smaller-sized mitochondria were extensively observed. Translocation of DRP1 and phosphorylation of its Ser-616 were significantly increased. Inhibition of mitochondrial fission with melatonin pretreatment significantly reduced cardiac function impairment [107]. Rat sepsis model induced by CLP showed significant decrease of MFN2 mRNA and increase of DRP1 mRNA, while there was MFN2 mRNA decrease in the endotoxemia model. Both models showed mitochondrial fragmentation in the heart [108]. Besides, serum from burn rats significantly increased mitochondrial fission in murine myoblast C2C12 cells, consistent with the decreased mitochondrial membrane potential and increased cell apoptosis. Treatment with IL-6 antibody prevented mitochondrial fragmentation and cell death, suggesting cytokine-induced mitochondrial fission plays an important role in second-hit tissue damage [109]. These studies suggest that abnormal fission-fusion balance may contribute to the progression of trauma-, burn-, and sepsis-induced organ function.

Mitochondrial biogenesis is also impaired in these acute pathologies. In LPS-treated hepatocytes [76], the septic heart [78], sepsis- or I/R-induced kidney injury [77, 110], etc., mitochondria mass was reduced, accompanied with deceased ATP production, decreased mitochondrial membrane potential, and increased oxidative injury and apoptosis. Decreased mitochondrial biogenesis and mitophagy caused by inhibition of SIRT1, PINK1, and Parkin are associated with higher risk of lung injury in sepsis [111]. Suppression of mitochondrial biogenesis through Toll-like receptor 4-dependent MAPK kinase increased endotoxin-induced acute kidney injury [112].

The behavior of mitophagy is complex. A sublethal dose of *E. coli* lipopolysaccharide (LPS) was injected to mouse; mitochondrial function was decreased temporarily and gets fully recovered later, but Parkin-deficient mice exhibited

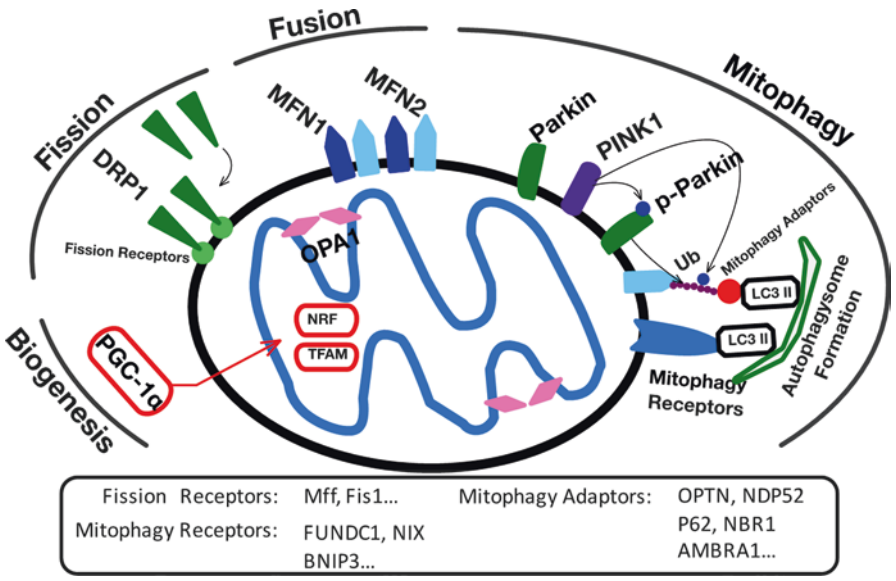

Fig. 4.2 Schematics for mitochondrial quality balance maintenance. Biogenesis: PGC-1α is the master of mitochondrial biogenesis, which activates NRF and TFAM in mitochondrial plasma to mediate mitochondrial protein synthesis. Fission: DRP1 is recruited from cytoplasm to bind receptors (e.g., MFF, FIS1) on mitochondrial outer membrane and hydrolyze GTP to drive mitochondrial fission. Fusion: MFN1 and MFN2 on mitochondrial outer membrane form homo-oligomers or hetero-oligomers and hydrolyze GTP to drive adjacent mitochondrial outer membrane fusion; OPA1 is responsible for inner membrane fusion and cristae morphology. Mitophagy: PINK1 is preserved on mitochondrial outer membrane and activates Parkin by phosphorylation, and then Parkin ubiquitin OMM proteins (e.g., MFN1, MFN2) and adaptors (e.g., OPTN, NDP52, p62, NBR1, etc.) are recruited, and then adaptors are connected to LC3 on autophagosome membrane; mitophagy receptors (e.g., FUNDC1, BNIP3, BNIP3L/NIX, etc.) on mitochondrial outer membrane interact with LC3 directly to mediate mitophagy

impaired recovery of cardiac contractility and constant degradation of mitochondrial metabolic functions, suggesting a protective role of mitophagy [113]. Ischemia/reperfusion injury is common in liver resection, hemorrhagic shock, and resuscitation. Due to nutrition and ATP depletion, calcium overload, and oxidative injury during ischemia, autophagy-related protein BECN1 and ATG7 are degraded, causing defective autophagy and mitochondrial clearance; more ROS production and CytC release were induced during fluid reperfusion and resulted in a severer liver injury, suggesting a harmful role of inadequate mitophagy [114]. By enhancing mitophagy, ischemic hepatocytes were protected from apoptosis [115], VSMC was prevented from LDL-induced injury [116], and lung epithelial cells were prevented from hypoxic injury [117]. However, the cross talk between autophagy and apoptosis makes the situation complex. A mouse model of cardiac myocyte ischemia/reperfusion injury showed increased autophagic flux, inhibiting autophagy

attenuated I/R-induced increase in oxidative stress, along with decrease in myocardial infarction size, suggesting that autophagy mediated myocardial injury during I/R [118]. However, whether mitophagy is a double-edged sword remains to be investigated.

4.3.3 Cross Talk of Fission, Fusion, Mitophagy, and Biogenesis Regulates Organ Function

Mitochondrial fission and fusion, mitophagy, and biogenesis are the dynamic process for maintaining the mitochondrial function. After fission, healthy mitochondria participate in normal function maintaining of cell, while slightly injured mitochondria can be restored with the help of fusion, severely damaged mitochondria with decreased membrane potential are degraded by mitophagy, and mitochondrial biogenesis counteracts the mitochondrial mass loss caused by mitochondrial clearance [119].

Studies showed that mitochondrial fission protein DRP1 may regulate mitochondrial fusion and mitophagy. The differential binding of MFN2 and DRP1 regulates mitochondrial fusion. DRP1 may act as a regulatory factor for both mitochondrial fusion and fission [120]. Studies found ablating DRP1 in myocytes not only interrupted the mitochondrial fission but also provoking mitophagy by regulating parkin [121], indicating a contrary role of fission and mitophagy.

The cross talk between fusion and mitophagy was also discovered. PINK1 may phosphorylate MFN2 at Thr-111 and Ser-442 and promote its Parkin-mediated ubiquitination and mitophagy [122]; ablation of either DRP1 or mitofusins (Mfn) in cardiomyocytes showed abnormal mitochondrial morphology; MFN null cells showed increased damaged mitochondria, while DRP1 null cells showed increased loss of mitochondria [123].

Mitochondrial biogenesis and mitophagy may cooperate to realize mitochondrial modification. Skeletal myoblasts may specifically shift from a highly glycolytic state to relying predominantly on oxidative phosphorylation (OXPHOS) upon differentiation; this phenomenon requires both mitochondrial clearance and biogenesis. During early myogenic differentiation, autophagy is robustly upregulated, and this coincides with mitophagy. Mitochondria are then repopulated via PPARGC1A/PGC-1alpha-mediated biogenesis; inhibiting autophagy may result in reduced mitochondrial biogenesis [58].

We may safely draw the conclusion that the regulation of mitochondrial fission, fusion, biogenesis, and mitophagy network has important influences on cell status and organ function. MODS is a consequence of various factors action, including infection, endotoxemia, hypoxia or ischemia, cytokines, etc. These factors may contribute to the impairment of one of the four processes (i.e., fission, fusion, biogenesis, and mitophagy). Multifactor-induced imbalance of mitochondrial quality may expand cell injury and participates in the occurrence of MODS (Fig. 4.3).

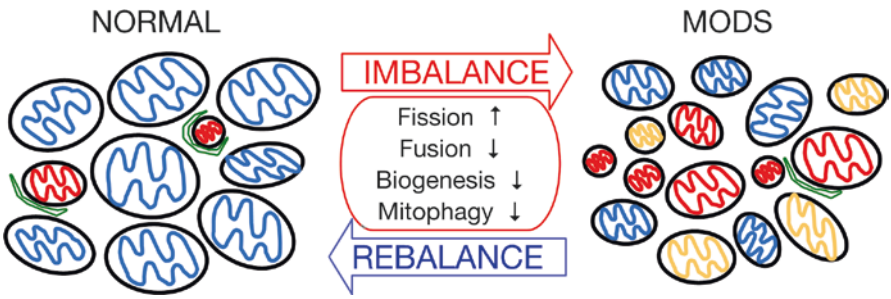

Fig. 4.3 Schematic for mitochondrial quality imbalance in MODS Mitochondria in normal cells undergo continuous fission, fusion, biogenesis, and mitophagy to maintain mitochondrial quality balance. Under stress conditions (e.g., severe trauma, shock, sepsis, MODS), the mitochondrial dynamics, biogenesis, and clearance are dysregulated and appear to be quality imbalance. Imbalanced mitochondrial quality may appear as mitochondrial fragmentation, decreased mitochondrial membrane potential and calcium overload, increased ROS and decreased ATP generation, etc. Restoring mitochondrial quality balance might be a therapeutic target to manage MODS. The blue inner mitochondrial membrane indicates a healthy status, the orange indicates unhealthy, and the red indicates damaged.

4.4 Restoring Mitochondrial Quality Balance Preserves Cellular and Organ Function

Some treatments have been applied to restore mitochondrial function such as vitamin C, vitamin E, β-carotene, coenzyme Q and resveratrol, and so on [124, 125]. Some novel antioxidants such as MitoQ, SS-31, MitoGSH, and MitoTEMPO have been developed and used to improve mitochondrial function. MitoQ, a mitochondria-targeted antioxidant, which is designed to protect against oxidative damage within mitochondria, has been proven to be beneficial for ischemic renal damage [126], intestinal inflammation [127], and sepsis [128]. SS-31 has gained benefits in neurodegeneration [129, 130] and hypoxic renal tubular cell injury [131] by decreasing free radical production and oxidative damage. MitoGSH may directly restore GSH levels and preserve mitochondrial redox buffering and signaling capacity [132]. Modifying mitochondrial respiration by manipulating substrate or electron transport chain enzyme activity are the other ways to improve mitochondrial function. A newly developed synthetic antimicrobial peptide 19-2.5 (Pep2.5) was found to prevent mitochondrial dysfunction in murine cardiomyocytes stimulated with serum from septic shock patients by enhancing the mitochondrial respiratory function and increasing cellular ATP levels [133]. In addition, other efforts aiming at decreasing calcium overload and apoptosis have also been made.

Large amount of studies observed the beneficial effect of modification of mitochondrial dynamics. Research showed inhibition of ROCK pathway with Fasudil significantly reduced the mitochondrial fragmentation in endotoxemic cardiomyocytes and improved the cardiac function [25]. Inhibition of the excessive

mitochondrial fission by blocking DRP1 with P110 at the onset of reperfusion significantly raised the long-term benefits of acute myocardial infarction [134]. Inhibition of mitochondrial fission with mdivi-1, the inhibitor of DRP1, significantly reduced Aβ-induced microglial apoptosis, exerting neuroprotective effect [135]. Mdivi-1 inhibition of mitochondrial fission may help to attenuate TBI-induced cell death through maintaining normal mitochondrial morphology and inhibiting activation of apoptosis [136]. In cardiomyocyte cell model of ischemia, overexpression of mitofusin proteins, MFN1 or MFN2, was found to be mitochondrial and cytoprotective [137].

Regulating mitochondrial biogenesis and PGC-1α expression was also found beneficial in some diseases including muscular, neurodegenerative disorders and renal and cardiac dysfunction [76, 110, 112, 138–141]. For example, overexpression of PINK1 and parkin could increase mitophagy of VSMC and decrease oxidized LDL-induced apoptosis [116].

4.5 Perspective

Mitochondrial quality imbalance might play a critical role in the occurrence and development of MODS. Severe trauma, shock, hypoxia, infection, and overproduction of inflammatory mediators and cytokines are the most common damage factors for mitochondrial quality and organ function. These factors can regulate or interrupt the balance of mitochondrial quality by regulating mitochondrial fission-, fusion-, mitophagy-, and biogenesis-related proteins such as DRP1, MFN1/MFN2, FIS1, OPA1, PGC1α, etc. [142–144].

p53 has been extensively studied in cancer and apoptosis; recent studies found p53 made a great contribution to directly modulating mitochondrial fission and fusion [145]. By attenuating the impairment of mitochondrial fission/fusion, biogenesis, and mitophagy, melatonin was found to be effective in restoring mitochondrial function in liver fibrosis induced by chronic carbon tetrachloride exposure [146]. Overexpression of heme oxygenase-1 (HO-1) may inhibit fission; promote fusion, biogenesis, and basal mitophagy; and thus play a protective role in heart oxidative injury [147]. Phospholipids in mitochondria are associated with dynamin-related GTPase (i.e., MFN1/MFN2, OPA1, DRP1), which regulate not only mitochondria fission and fusion but also mitophagy [148]. These studies suggested that manipulating mitochondrial fission and fusion, biogenesis, and mitophagy simultaneously seems to be more effective in correcting mitochondrial quality imbalance in some complicated situations.

Endoplasmic reticulum (ER) and mitochondria contact were considered a transport platform for calcium, the lipid between mitochondria and ER. Recent study found mitochondrial fission occurred at the mito-ER contact site. This contact may regulate mitochondrial fission, fusion, and mitophagy [149]. Studies are called to examine the exact role of such contact, which may inspire new therapeutic measures for mitochondrial imbalance and organ function protection.

References

1. Ulvik A, Kvale R, Wentzel-Larsen T, Flaatten H. Multiple organ failure after trauma affects even long-term survival and functional status. Crit Care. 2007;11(5):R95.
2. Santulli G, Xie W, Reiken SR, Marks AR. Mitochondrial calcium overload is a key determinant in heart failure. Proc Natl Acad Sci U S A. 2015;112(36):11389–94.
3. Li X, Fang P, Mai J, Choi ET, Wang H, Yang XF. Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. J Hematol Oncol. 2013;6:19.
4. Rossier MF. T channels and steroid biosynthesis: in search of a link with mitochondria. Cell Calcium. 2006;40(2):155–64.
5. Jang DH, Greenwood JC, Spyres MB, Eckmann DM. Measurement of mitochondrial respiration and motility in acute care: sepsis, trauma, and poisoning. J Intensive Care Med. 2017;32(1):86–94.
6. Scheibye-Knudsen M, Fang EF, Croteau DL, Wilson DM 3rd, Bohr VA. Protecting the mitochondrial powerhouse. Trends Cell Biol. 2015;25(3):158–70.
7. Marchi S, Pinton P. The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. J Physiol. 2014;592(5):829–39.
8. Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM. p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell. 2012;149(7):1536–48.
9. Volgyi K, Juhasz G, Kovacs Z, Penke B. Dysfunction of endoplasmic reticulum (ER) and mitochondria (MT) in Alzheimer's disease: the role of the ER-MT cross-talk. Curr Alzheimer Res. 2015;12(7):655–72.
10. Schapira AH, Olanow CW, Greenamyre JT, Bezard E. Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. Lancet. 2014;384(9942):545–55.
11. Wu CC, Bratton SB. Regulation of the intrinsic apoptosis pathway by reactive oxygen species. Antioxid Redox Signal. 2013;19(6):546–58.
12. Busch KB, Kowald A, Spelbrink JN. Quality matters: how does mitochondrial network dynamics and quality control impact on mtDNA integrity? Philos Trans R Soc Lond Ser B Biol Sci. 2014;369(1646):20130442.
13. Wang L, Ishihara T, Ibayashi Y, Tatsushima K, Setoyama D, Hanada Y, Takeichi Y, Sakamoto S, Yokota S, Mihara K, Kang D, Ishihara N, Takayanagi R, Nomura M. Disruption of mitochondrial fission in the liver protects mice from diet-induced obesity and metabolic deterioration. Diabetologia. 2015;58(10):2371–80.
14. Hernandez-Alvarez MI, Paz JC, Sebastian D, Munoz JP, Liesa M, Segales J, Palacin M, Zorzano A. Glucocorticoid modulation of mitochondrial function in hepatoma cells requires the mitochondrial fission protein DRP1. Antioxid Redox Signal. 2013;19(4):366–78.
15. Roth D, Krammer PH, Gulow K. Dynamin related protein 1-dependent mitochondrial fission regulates oxidative signalling in T cells. FEBS Lett. 2014;588(9):1749–54.
16. Richter V, Palmer CS, Osellame LD, Singh AP, Elgass K, Stroud DA, Sesaki H, Kvansakul M, Ryan MT. Structural and functional analysis of MiD51, a dynamin receptor required for mitochondrial fission. J Cell Biol. 2014;204(4):477–86.
17. Hatch AL, Gurel PS, Higgs HN. Novel roles for actin in mitochondrial fission. J Cell Sci. 2014;127(Pt 21):4549–60.
18. Korobova F, Gauvin TJ, Higgs HN. A role for myosin II in mammalian mitochondrial fission. Curr Biol. 2014;24(4):409–14.
19. Otera H, Ishihara N, Mihara K. New insights into the function and regulation of mitochondrial fission. Biochim Biophys Acta. 2013;1833(5):1256–68.
20. Godoy JA, Arrazola MS, Ordenes D, Silva-Alvarez C, Braidy N, Inestrosa NC. Wnt-5a ligand modulates mitochondrial fission-fusion in rat hippocampal neurons. J Biol Chem. 2014;289(52):36179–93.

21. Liang N, Wang P, Wang S, Li S, Li Y, Wang J, Wang M. Role of mitochondrial calcium uniporter in regulating mitochondrial fission in the cerebral cortexes of living rats. J Neural Transm (Vienna). 2014;121(6):593–600.
22. Pennanen C, Parra V, Lopez-Crisosto C, Morales PE, Del Campo A, Gutierrez T, Rivera-Mejias P, Kuzmicic J, Chiong M, Zorzano A, Rothermel BA, Lavandero S. Mitochondrial fission is required for cardiomyocyte hypertrophy mediated by a Ca2+-calcineurin signaling pathway. J Cell Sci. 2014;127(Pt 12):2659–71.
23. Chen SD, Lin TK, Yang DI, Lee SY, Shaw FZ, Liou CW, Chuang YC. Roles of PTEN-induced putative kinase 1 and dynamin-related protein 1 in transient global ischemia-induced hippocampal neuronal injury. Biochem Biophys Res Commun. 2015;460(2):397–403.
24. Buhlman L, Damiano M, Bertolin G, Ferrando-Miguel R, Lombes A, Brice A, Corti O. Functional interplay between Parkin and DRP1 in mitochondrial fission and clearance. Biochim Biophys Acta. 2014;1843(9):2012–26.
25. Preau S, Delguste F, Yu Y, Remy-Jouet I, Richard V, Saulnier F, Boulanger E, Neviere R. Endotoxemia engages the RhoA kinase pathway to impair cardiac function by altering cytoskeleton, mitochondrial fission, and autophagy. Antioxid Redox Signal. 2016;24(10):529–42.
26. Wang W, Wang Y, Long J, Wang J, Haudek SB, Overbeek P, Chang BH, Schumacker PT, Danesh FR. Mitochondrial fission triggered by hyperglycemia is mediated by ROCK1 activation in podocytes and endothelial cells. Cell Metab. 2012;15(2):186–200.
27. Prieto J, Leon M, Ponsoda X, Sendra R, Bort R, Ferrer-Lorente R, Raya A, Lopez-Garcia C, Torres J. Early ERK1/2 activation promotes DRP1-dependent mitochondrial fission necessary for cell reprogramming. Nat Commun. 2016;7:11124.
28. Kim B, Park J, Chang KT, Lee DS. Peroxiredoxin 5 prevents amyloid-beta oligomer-induced neuronal cell death by inhibiting ERK-DRP1-mediated mitochondrial fragmentation. Free Radic Biol Med. 2016;90:184–94.
29. Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K. Mitotic phosphorylation of dynamin-related GTPase DRP1 participates in mitochondrial fission. J Biol Chem. 2007;282(15):11521–9.
30. Jahani-Asl A, Huang E, Irrcher I, Rashidian J, Ishihara N, Lagace DC, Slack RS, Park DS. CDK5 phosphorylates DRP1 and drives mitochondrial defects in NMDA-induced neuronal death. Hum Mol Genet. 2015;24(16):4573–83.
31. Nakamura N, Kimura Y, Tokuda M, Honda S, Hirose S. MARCH-V is a novel mitofusin 2- and DRP1-binding protein able to change mitochondrial morphology. EMBO Rep. 2006;7(10):1019–22.
32. Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, Ohmura-Hoshino M, Sada K, Hotta H, Yamamura H, Inatome R, Yanagi S. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. EMBO J. 2006;25(15):3618–26.
33. Xu S, Cherok E, Das S, Li S, Roelofs BA, Ge SX, Polster BM, Boyman L, Lederer WJ, Wang C, Karbowski M. Mitochondrial E3 ubiquitin ligase MARCH5 controls mitochondrial fission and cell sensitivity to stress-induced apoptosis through regulation of MiD49 protein. Mol Biol Cell. 2016;27(2):349–59.
34. Cho DH, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z, Lipton SA. S-nitrosylation of DRP1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. Science. 2009;324(5923):102–5.
35. Braschi E, Zunino R, Mcbride HM. MAPL is a new mitochondrial SUMO E3 ligase that regulates mitochondrial fission. EMBO Rep. 2009;10(7):748–54.
36. Zunino R, Schauss A, Rippstein P, Andrade-Navarro M, Mcbride HM. The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. J Cell Sci. 2007;120(Pt 7):1178–88.
37. Gawlowski T, Suarez J, Scott B, Torres-Gonzalez M, Wang H, Schwappacher R, Han X, Yates JR 3rd, Hoshijima M, Dillmann W. Modulation of dynamin-related protein 1 (DRP1) function by increased O-linked-beta-N-acetylglucosamine modification (O-GlcNAc) in cardiac myocytes. J Biol Chem. 2012;287(35):30024–34.

38. Yim N, Ryu SW, Han EC, Yoon J, Choi K, Choi C. Mutant ubiquitin UBB+1 induces mitochondrial fusion by destabilizing mitochondrial fission-specific proteins and confers resistance to oxidative stress-induced cell death in astrocytic cells. PLoS One. 2014;9(6):e99937.
39. Kasahara A, Cipolat S, Chen Y, Dorn GW 2nd, Scorrano L. Mitochondrial fusion directs cardiomyocyte differentiation via calcineurin and Notch signaling. Science. 2013;342(6159):734–7.
40. Ballweg K, Mutze K, Konigshoff M, Eickelberg O, Meiners S. Cigarette smoke extract affects mitochondrial function in alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2014;307(11):L895–907.
41. Hoppins S, Nunnari J. The molecular mechanism of mitochondrial fusion. Biochim Biophys Acta. 2009;1793(1):20–6.
42. Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, Lenaers G. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem. 2003;278(10):7743–6.
43. Merkwirth C, Dargazanli S, Tatsuta T, Geimer S, Lower B, Wunderlich FT, Von Kleist-Retzow JC, Waisman A, Westermann B, Langer T. Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. Genes Dev. 2008;22(4):476–88.
44. Ranieri M, Brajkovic S, Riboldi G, Ronchi D, Rizzo F, Bresolin N, Corti S, Comi GP. Mitochondrial fusion proteins and human diseases. Neurol Res Int. 2013;2013:293893.
45. Duarte A, Castillo AF, Podesta EJ, Poderoso C. Mitochondrial fusion and ERK activity regulate steroidogenic acute regulatory protein localization in mitochondria. PLoS One. 2014;9(6):e100387.
46. Hickey FB, Corcoran JB, Griffin B, Bhreathnach U, Mortiboys H, Reid HM, Andrews D, Byrne S, Furlong F, Martin F, Godson C, Murphy M. IHG-1 increases mitochondrial fusion and bioenergetic function. Diabetes. 2014;63(12):4314–25.
47. Silvander JSG, Kvarnstrom SM, Kumari-Ilieva A, Shrestha A, Alam CM, Toivola DM. Keratins regulate beta-cell mitochondrial morphology, motility, and homeostasis. FASEB J. 2017;31:4578.
48. Martorell-Riera A, Segarra-Mondejar M, Munoz JP, Ginet V, Olloquequi J, Perez-Clausell J, Palacin M, Reina M, Puyal J, Zorzano A, Soriano FX. MFN2 downregulation in excitotoxicity causes mitochondrial dysfunction and delayed neuronal death. EMBO J. 2014;33(20):2388–407.
49. Lee JY, Kapur M, Li M, Choi MC, Choi S, Kim HJ, Kim I, Lee E, Taylor JP, Yao TP. MFN1 deacetylation activates adaptive mitochondrial fusion and protects metabolically challenged mitochondria. J Cell Sci. 2014;127(Pt 22):4954–63.
50. Glauser L, Sonnay S, Stafa K, Moore DJ. Parkin promotes the ubiquitination and degradation of the mitochondrial fusion factor mitofusin 1. J Neurochem. 2011;118(4):636–45.
51. Yue W, Chen Z, Liu H, Yan C, Chen M, Feng D, Yan C, Wu H, Du L, Wang Y, Liu J, Huang X, Xia L, Liu L, Wang X, Jin H, Wang J, Song Z, Hao X, Chen Q. A small natural molecule promotes mitochondrial fusion through inhibition of the deubiquitinase USP30. Cell Res. 2014;24(4):482–96.
52. Parra V, Verdejo HE, Iglewski M, Del Campo A, Troncoso R, Jones D, Zhu Y, Kuzmicic J, Pennanen C, Lopez-Crisosto C, Jana F, Ferreira J, Noguera E, Chiong M, Bernlohr DA, Klip A, Hill JA, Rothermel BA, Abel ED, Zorzano A, Lavandero S. Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFkappaB-Opa-1 signaling pathway. Diabetes. 2014;63(1):75–88.
53. Zhang L, He Z, Zhang Q, Wu Y, Yang X, Niu W, Hu Y, Jia J. Exercise pretreatment promotes mitochondrial dynamic protein OPA1 expression after cerebral ischemia in rats. Int J Mol Sci. 2014;15(3):4453–63.
54. Song Z, Chen H, Fiket M, Alexander C, Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J Cell Biol. 2007;178(5):749–55.
55. Griparic L, Kanazawa T, Van Der Bliek AM. Regulation of the mitochondrial dynamin-like protein OPA1 by proteolytic cleavage. J Cell Biol. 2007;178(5):757–64.

56. Quiros PM, Ramsay AJ, Sala D, Fernandez-Vizarra E, Rodriguez F, Peinado JR, Fernandez-Garcia MS, Vega JA, Enriquez JA, Zorzano A, Lopez-Otin C. Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice. EMBO J. 2012;31(9):2117–33.
57. Anand R, Wai T, Baker MJ, Kladt N, Schauss AC, Rugarli E, Langer T. The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J Cell Biol. 2014;204(6):919–29.
58. Sin J, Andres AM, Taylor DJ, Weston T, Hiraumi Y, Stotland A, Kim BJ, Huang C, Doran KS, Gottlieb RA. Mitophagy is required for mitochondrial biogenesis and myogenic differentiation of C2C12 myoblasts. Autophagy. 2016;12(2):369–80.
59. Glick D, Zhang W, Beaton M, Marsboom G, Gruber M, Simon MC, Hart J, Dorn GW 2nd, Brady MJ, Macleod KF. BNip3 regulates mitochondrial function and lipid metabolism in the liver. Mol Cell Biol. 2012;32(13):2570–84.
60. Soleimanpour SA, Gupta A, Bakay M, Ferrari AM, Groff DN, Fadista J, Spruce LA, Kushner JA, Groop L, Seeholzer SH, Kaufman BA, Hakonarson H, Stoffers DA. The diabetes susceptibility gene Clec16a regulates mitophagy. Cell. 2014;157(7):1577–90.
61. Tan T, Zimmermann M, Reichert AS. Controlling quality and amount of mitochondria by mitophagy: insights into the role of ubiquitination and deubiquitination. Biol Chem. 2016;397(7):637–47.
62. Yamaguchi O, Murakawa T, Nishida K, Otsu K. Receptor-mediated mitophagy. J Mol Cell Cardiol. 2016;95:50–6.
63. Hamacher-Brady A, Brady NR. Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. Cell Mol Life Sci. 2016;73(4):775–95.
64. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochim Biophys Acta. 2011;1813(7):1269–78.
65. Wenz T. Regulation of mitochondrial biogenesis and PGC-1alpha under cellular stress. Mitochondrion. 2013;13(2):134–42.
66. Fernandez-Vizarra E, Enriquez JA, Perez-Martos A, Montoya J, Fernandez-Silva P. Mitochondrial gene expression is regulated at multiple levels and differentially in the heart and liver by thyroid hormones. Curr Genet. 2008;54(1):13–22.
67. Chen JQ, Cammarata PR, Baines CP, Yager JD. Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. Biochim Biophys Acta. 2009;1793(10):1540–70.
68. Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, Ke H, Rehmann H, Taussig R, Brown AL, Kim MK, Beaven MA, Burgin AB, Manganiello V, Chung JH. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. Cell. 2012;148(3):421–33.
69. Le Pennec S, Mirebeau-Prunier D, Boutet-Bouzamondo N, Jacques C, Guillotin D, Lauret E, Houlgatte R, Malthiery Y, Savagner F. Nitric oxide and calcium participate in the fine regulation of mitochondrial biogenesis in follicular thyroid carcinoma cells. J Biol Chem. 2011;286(20):18229–39.
70. Santandreu FM, Oliver J, Roca P. Improvement of mitochondrial energy and oxidative balance during intestinal differentiation. Mitochondrion. 2011;11(1):89–96.
71. Rogers RP, Rogina B. Increased mitochondrial biogenesis preserves intestinal stem cell homeostasis and contributes to longevity in Indy mutant flies. Aging (Albany NY). 2014;6(4):335–50.
72. D'Errico I, Salvatore L, Murzilli S, Lo Sasso G, Latorre D, Martelli N, Egorova AV, Polishuck R, Madeyski-Bengtson K, Lelliott C, Vidal-Puig AJ, Seibel P, Villani G, Moschetta A. Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1alpha) is a metabolic regulator of intestinal epithelial cell fate. Proc Natl Acad Sci U S A. 2011;108(16):6603–8.
73. Llimona F, De Lima TM, Moretti AI, Theobaldo M, Jukemura J, Velasco IT, Machado MC, Souza HP. PGC-1alpha expression is increased in leukocytes in experimental acute pancreatitis. Inflammation. 2014;37(4):1231–9.

74. Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. Nat Immunol. 2017;18(5):488–98.
75. Yang CS, Kim JJ, Lee HM, Jin HS, Lee SH, Park JH, Kim SJ, Kim JM, Han YM, Lee MS, Kweon GR, Shong M, Jo EK. The AMPK-PPARGC1A pathway is required for antimicrobial host defense through activation of autophagy. Autophagy. 2014;10(5):785–802.
76. Xing W, Yang L, Peng Y, Wang Q, Gao M, Yang M, Xiao X. Ginsenoside Rg3 attenuates sepsis-induced injury and mitochondrial dysfunction in liver via AMPK-mediated autophagy flux. Biosci Rep. 2017;37(4):BSR20170934.
77. Parikh SM, Yang Y, He L, Tang C, Zhan M, Dong Z. Mitochondrial function and disturbances in the septic kidney. Semin Nephrol. 2015;35(1):108–19.
78. Alvarez S, Vico T, Vanasco V. Cardiac dysfunction, mitochondrial architecture, energy production, and inflammatory pathways: interrelated aspects in endotoxemia and sepsis. Int J Biochem Cell Biol. 2016;81(Pt B):307–14.
79. Suliman HB, Kraft BD, Bartz RR, Chen L, Welty-Wolf KE, Piantadosi CA. Mitochondrial quality control in alveolar epithelial cells damaged by S. aureus pneumonia in mice. Am J Physiol Lung Cell Mol Physiol. 2017;31:L699.
80. Wu H, Wei H, Sehgal SA, Liu L, Chen Q. Mitophagy receptors sense stress signals and couple mitochondrial dynamic machinery for mitochondrial quality control. Free Radic Biol Med. 2016;100:199–209.
81. Valero T. Mitochondrial biogenesis: pharmacological approaches. Curr Pharm Des. 2014;20(35):5507–9.
82. Rub C, Wilkening A, Voos W. Mitochondrial quality control by the Pink1/Parkin system. Cell Tissue Res. 2017;367(1):111–23.
83. Bondi H, Zilocchi M, Mare MG, D'Agostino G, Giovannardi S, Ambrosio S, Fasano M, Alberio T. Dopamine induces mitochondrial depolarization without activating PINK1-mediated mitophagy. J Neurochem. 2016;136:1219.
84. Rojas-Charry L, Cookson MR, Nino A, Arboleda H, Arboleda G. Downregulation of Pink1 influences mitochondrial fusion-fission machinery and sensitizes to neurotoxins in dopaminergic cells. Neurotoxicology. 2014;44:140–8.
85. Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, Zhu X. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. J Neurochem. 2012;120(3):419–29.
86. Baltrusch S. Mitochondrial network regulation and its potential interference with inflammatory signals in pancreatic beta cells. Diabetologia. 2016;59(4):683–7.
87. Joseph AM, Joanisse DR, Baillot RG, Hood DA. Mitochondrial dysregulation in the pathogenesis of diabetes: potential for mitochondrial biogenesis-mediated interventions. Exp Diabetes Res. 2012;2012:642038.
88. Mcgill JK, Beal MF. PGC-1alpha, a new therapeutic target in Huntington's disease? Cell. 2006;127(3):465–8.
89. Chen TT, Wu LS, Hsu PW, Pang CY, Lee KM, Cheng PC, Peng SY. Mitochondrial dynamics in the mouse liver infected by Schistosoma mansoni. Acta Trop. 2015;148:13–23.
90. Yu T, Wang L, Lee H, O'Brien DK, Bronk SF, Gores GJ, Yoon Y. Decreasing mitochondrial fission prevents cholestatic liver injury. J Biol Chem. 2014;289(49):34074–88.
91. Zhang X, Shan P, Homer R, Zhang Y, Petrache I, Mannam P, Lee PJ. Cathepsin E promotes pulmonary emphysema via mitochondrial fission. Am J Pathol. 2014;184(10):2730–41.
92. Lu J, Shen Y, Liu LJ, Qian HY, Zhu CL. Combining epinephrine and esmolol attenuates excessive autophagy and mitophagy in rat cardiomyocytes after cardiac arrest. J Cardiovasc Pharmacol. 2015;66(5):449–56.
93. Chen Y, Liu Y, Dorn GW 2nd. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. Circ Res. 2011;109(12):1327–31.
94. Papanicolaou KN, Kikuchi R, Ngoh GA, Coughlan KA, Dominguez I, Stanley WC, Walsh K. Mitofusins 1 and 2 are essential for postnatal metabolic remodeling in heart. Circ Res. 2012;111(8):1012–26.

95. Joshi SR, Dhagia V, Gairhe S, Edwards JG, Mcmurtry IF, Gupte SA. MicroRNA-140 is elevated and mitofusin-1 is downregulated in the right ventricle of the Sugen5416/hypoxia/normoxia model of pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol. 2016;311(3):H689–98.
96. Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. Virulence. 2014;5(1):66–72.
97. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet. 2002;360(9328):219–23.
98. Carre JE, Orban JC, Re L, Felsmann K, Iffert W, Bauer M, Suliman HB, Piantadosi CA, Mayhew TM, Breen P, Stotz M, Singer M. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med. 2010;182(6):745–51.
99. Karlsson M, Hara N, Morata S, Sjovall F, Kilbaugh T, Hansson MJ, Uchino H, Elmer E. Diverse and tissue-specific mitochondrial respiratory response in a mouse model of sepsis-induced multiple organ failure. Shock. 2016;45(4):404–10.
100. Neviere R, Delguste F, Durand A, Inamo J, Boulanger E, Preau S. Abnormal mitochondrial cAMP/PKA signaling is involved in sepsis-induced mitochondrial and myocardial dysfunction. Int J Mol Sci. 2016;17(12):E2075.
101. Jeger V, Brandt S, Porta F, Jakob SM, Takala J, Djafarzadeh S. Dose response of endotoxin on hepatocyte and muscle mitochondrial respiration in vitro. Biomed Res Int. 2015;2015:353074.
102. Herminghaus A, Barthel F, Heinen A, Beck C, Vollmer C, Bauer I, Weidinger A, Kozlov AV, Picker O. Severity of polymicrobial sepsis modulates mitochondrial function in rat liver. Mitochondrion. 2015;24:122–8.
103. Joseph LC, Kokkinaki D, Valenti MC, Kim GJ, Barca E, Tomar D, Hoffman NE, Subramanyam P, Colecraft HM, Hirano M, Ratner AJ, Madesh M, Drosatos K, Morrow JP. Inhibition of NADPH oxidase 2 (NOX2) prevents sepsis-induced cardiomyopathy by improving calcium handling and mitochondrial function. JCI Insight. 2017;2(17):94248.
104. Stoyanoff TR, Todaro JS, Aguirre MV, Zimmermann MC, Brandan NC. Amelioration of lipopolysaccharide-induced acute kidney injury by erythropoietin: involvement of mitochondria-regulated apoptosis. Toxicology. 2014;318:13–21.
105. Yi L, Huang X, Guo F, Zhou Z, Chang M, Tang J, Huan J. Lipopolysaccharide induces human pulmonary micro-vascular endothelial apoptosis via the YAP signaling pathway. Front Cell Infect Microbiol. 2016;6:133.
106. Kautza B, Gomez H, Escobar D, Corey C, Ataya B, Luciano J, Botero AM, Gordon L, Brumfield J, Martinez S, Holder A, Ogundele O, Pinsky M, Shiva S, Zuckerbraun BS. Inhaled, nebulized sodium nitrite protects in murine and porcine experimental models of hemorrhagic shock and resuscitation by limiting mitochondrial injury. Nitric Oxide. 2015;51:7–18.
107. Ding M, Ning J, Feng N, Li Z, Liu Z, Wang Y, Wang Y, Li X, Huo C, Jia X, Xu R, Fu F, Wang X, Pei J. Dynamin-related protein 1-mediated mitochondrial fission contributes to post-traumatic cardiac dysfunction in rats and the protective effect of melatonin. J Pineal Res. 2017;64:e12447.
108. Gonzalez AS, Elguero ME, Finocchietto P, Holod S, Romorini L, Miriuka SG, Peralta JG, Poderoso JJ, Carreras MC. Abnormal mitochondrial fusion-fission balance contributes to the progression of experimental sepsis. Free Radic Res. 2014;48(7):769–83.
109. Sehat A, Huebinger RM, Carlson DL, Zang QS, Wolf SE, Song J. Burn serum stimulates myoblast cell death associated with IL-6-induced mitochondrial fragmentation. Shock. 2017;48(2):236–42.
110. Jesinkey SR, Funk JA, Stallons LJ, Wills LP, Megyesi JK, Beeson CC, Schnellmann RG. Formoterol restores mitochondrial and renal function after ischemia-reperfusion injury. J Am Soc Nephrol. 2014;25(6):1157–62.
111. Mannam P, Shinn AS, Srivastava A, Neamu RF, Walker WE, Bohanon M, Merkel J, Kang MJ, Dela Cruz CS, Ahasic AM, Pisani MA, Trentalange M, West AP, Shadel GS, Elias JA, Lee PJ. MKK3 regulates mitochondrial biogenesis and mitophagy in sepsis-induced lung injury. Am J Physiol Lung Cell Mol Physiol. 2014;306(7):L604–19.

112. Smith JA, Stallons LJ, Collier JB, Chavin KD, Schnellmann RG. Suppression of mitochondrial biogenesis through toll-like receptor 4-dependent mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signaling in endotoxin-induced acute kidney injury. J Pharmacol Exp Ther. 2015;352(2):346–57.
113. Piquereau J, Godin R, Deschenes S, Bessi VL, Mofarrahi M, Hussain SN, Burelle Y. Protective role of PARK2/Parkin in sepsis-induced cardiac contractile and mitochondrial dysfunction. Autophagy. 2013;9(11):1837–51.
114. Go KL, Lee S, Zendejas I, Behrns KE, Kim JS. Mitochondrial dysfunction and autophagy in hepatic ischemia/reperfusion injury. Biomed Res Int. 2015;2015:183469.
115. Wang K. Autophagy and apoptosis in liver injury. Cell Cycle. 2015;14(11):1631–42.
116. Swiader A, Nahapetyan H, Faccini J, D'Angelo R, Mucher E, Elbaz M, Boya P, Vindis C. Mitophagy acts as a safeguard mechanism against human vascular smooth muscle cell apoptosis induced by atherogenic lipids. Oncotarget. 2016;7(20):28821–35.
117. Liang X, Wei SQ, Lee SJ, Fung JK, Zhang M, Tanaka A, Choi AM, Jin Y. p62 sequestosome 1/light chain 3b complex confers cytoprotection on lung epithelial cells after hyperoxia. Am J Respir Cell Mol Biol. 2013;48(4):489–96.
118. Gao L, Jiang T, Guo J, Liu Y, Cui G, Gu L, Su L, Zhang Y. Inhibition of autophagy contributes to ischemic postconditioning-induced neuroprotection against focal cerebral ischemia in rats. PLoS One. 2012;7(9):e46092.
119. Yang JY, Yang WY. Bit-by-bit autophagic removal of parkin-labelled mitochondria. Nat Commun. 2013;4:2428.
120. Huang P, Galloway CA, Yoon Y. Control of mitochondrial morphology through differential interactions of mitochondrial fusion and fission proteins. PLoS One. 2011;6(5):e20655.
121. Song M, Gong G, Burelle Y, Gustafsson AB, Kitsis RN, Matkovich SJ, Dorn GW 2nd. Interdependence of Parkin-mediated mitophagy and mitochondrial fission in adult mouse hearts. Circ Res. 2015;117(4):346–51.
122. Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science. 2013;340(6131):471–5.
123. Song M, Mihara K, Chen Y, Scorrano L, Dorn GW 2nd. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. Cell Metab. 2015;21(2):273–85.
124. Zhang B, Xu L, Zhuo N, Shen J. Resveratrol protects against mitochondrial dysfunction through autophagy activation in human nucleus pulposus cells. Biochem Biophys Res Commun. 2017;493(1):373–81.
125. Muthulakshmi S, Saravanan R. Protective effects of azelaic acid against high-fat diet-induced oxidative stress in liver, kidney and heart of C57BL/6J mice. Mol Cell Biochem. 2013;377(1-2):23–33.
126. Dietl A, Maack C. Targeting mitochondrial calcium handling and reactive oxygen species in heart failure. Curr Heart Fail Rep. 2017;14(4):338–49.
127. Formentini L, Santacatterina F, Nunez De Arenas C, Stamatakis K, Lopez-Martinez D, Logan A, Fresno M, Smits R, Murphy MP, Cuezva JM. Mitochondrial ROS production protects the intestine from inflammation through functional M2 macrophage polarization. Cell Rep. 2017;19(6):1202–13.
128. Prauchner CA. Oxidative stress in sepsis: pathophysiological implications justifying antioxidant co-therapy. Burns. 2017;43(3):471–85.
129. Reddy PH, Manczak M, Kandimalla R. Mitochondria-targeted small molecule SS31: a potential candidate for the treatment of Alzheimer's disease. Hum Mol Genet. 2017;26(8):1483–96.
130. Yin X, Manczak M, Reddy PH. Mitochondria-targeted molecules MitoQ and SS31 reduce mutant huntingtin-induced mitochondrial toxicity and synaptic damage in Huntington's disease. Hum Mol Genet. 2016;25(9):1739–53.
131. Zhao WY, Han S, Zhang L, Zhu YH, Wang LM, Zeng L. Mitochondria-targeted antioxidant peptide SS31 prevents hypoxia/reoxygenation-induced apoptosis by down-regulating p66Shc in renal tubular epithelial cells. Cell Physiol Biochem. 2013;32(3):591–600.

132. Mailloux RJ. Application of mitochondria-targeted pharmaceuticals for the treatment of heart disease. Curr Pharm Des. 2016;22(31):4763–79.
133. Martin L, Peters C, Heinbockel L, Moellmann J, Martincuks A, Brandenburg K, Lehrke M, Muller-Newen G, Marx G, Schuerholz T. The synthetic antimicrobial peptide 19-2.5 attenuates mitochondrial dysfunction in cardiomyocytes stimulated with human sepsis serum. Innate Immun. 2016;22(8):612–9.
134. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X, Mochly-Rosen D. Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. J Am Heart Assoc. 2013;2(5):e000461.
135. Xie N, Wang C, Lian Y, Wu C, Zhang H, Zhang Q. Inhibition of mitochondrial fission attenuates Abeta-induced microglia apoptosis. Neuroscience. 2014;256:36–42.
136. Wu Q, Xia SX, Li QQ, Gao Y, Shen X, Ma L, Zhang MY, Wang T, Li YS, Wang ZF, Luo CL, Tao LY. Mitochondrial division inhibitor 1 (Mdivi-1) offers neuroprotection through diminishing cell death and improving functional outcome in a mouse model of traumatic brain injury. Brain Res. 2016;1630:134–43.
137. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010;121(18):2012–22.
138. Dillon LM, Hida A, Garcia S, Prolla TA, Moraes CT. Long-term bezafibrate treatment improves skin and spleen phenotypes of the mtDNA mutator mouse. PLoS One. 2012;7(9):e44335.
139. Dillon LM, Williams SL, Hida A, Peacock JD, Prolla TA, Lincoln J, Moraes CT. Increased mitochondrial biogenesis in muscle improves aging phenotypes in the mtDNA mutator mouse. Hum Mol Genet. 2012;21(10):2288–97.
140. Mudo G, Makela J, Di Liberto V, Tselykh TV, Olivieri M, Piepponen P, Eriksson O, Malkia A, Bonomo A, Kairisalo M, Aguirre JA, Korhonen L, Belluardo N, Lindholm D. Transgenic expression and activation of PGC-1alpha protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. Cell Mol Life Sci. 2012;69(7):1153–65.
141. Mccreath G, Scullion MM, Lowes DA, Webster NR, Galley HF. Pharmacological activation of endogenous protective pathways against oxidative stress under conditions of sepsis. Br J Anaesth. 2016;116(1):131–9.
142. Rozova EV, Mankovskaya IN, Mironova GD. Structural and dynamic changes in mitochondria of rat myocardium under acute hypoxic hypoxia: role of mitochondrial ATP-dependent potassium channel. Biochemistry (Mosc). 2015;80(8):994–1000.
143. Sanderson TH, Raghunayakula S, Kumar R. Neuronal hypoxia disrupts mitochondrial fusion. Neuroscience. 2015;301:71–8.
144. Anusree SS, Nisha VM, Priyanka A, Raghu KG. Insulin resistance by TNF-alpha is associated with mitochondrial dysfunction in 3T3-L1 adipocytes and is ameliorated by punicic acid, a PPARgamma agonist. Mol Cell Endocrinol. 2015;413:120–8.
145. Wang DB, Kinoshita C, Kinoshita Y, Morrison RS. p53 and mitochondrial function in neurons. Biochim Biophys Acta. 2014;1842(8):1186–97.
146. Kang JW, Hong JM, Lee SM. Melatonin enhances mitophagy and mitochondrial biogenesis in rats with carbon tetrachloride-induced liver fibrosis. J Pineal Res. 2016;60(4):383–93.
147. Hull TD, Boddu R, Guo L, Tisher CC, Traylor AM, Patel B, Joseph R, Prabhu SD, Suliman HB, Piantadosi CA, Agarwal A, George JF. Heme oxygenase-1 regulates mitochondrial quality control in the heart. JCI Insight. 2016;1(2):e85817.
148. Zhang Q, Tamura Y, Roy M, Adachi Y, Iijima M, Sesaki H. Biosynthesis and roles of phospholipids in mitochondrial fusion, division and mitophagy. Cell Mol Life Sci. 2014;71(19):3767–78.
149. Wu W, Lin C, Wu K, Jiang L, Wang X, Li W, Zhuang H, Zhang X, Chen H, Li S, Yang Y, Lu Y, Wang J, Zhu R, Zhang L, Sui S, Tan N, Zhao B, Zhang J, Li L, Feng D. FUNDC1 regulates mitochondrial dynamics at the ER-mitochondrial contact site under hypoxic conditions. EMBO J. 2016;35(13):1368–84.

Lymph Formation and Transport: Role in Trauma-Hemorrhagic Shock

5

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Abstract

Microcirculatory disturbance is the key line of multiple organ dysfunction and failure following trauma-hemorrhagic shock (T/HS). The lymphatic circulation is an important component of the circulatory system, which is involved in the pathogenesis of T/HS. In the early stage of T/HS, the enhanced lymphatic constriction and reactivity, paralleling with the increased lymph formation and transport, play an important compensatory role in alleviating tissue edema and organ injury. Afterward, along with the continuation of hypotension, the lymphatic contractility and reactivity are reduced that causes tissue edema and organ injury. Moreover, T/HS-induced ischemia and/or reperfusion result in intestinal barrier injury and lymphatic endothelial barrier dysfunction. Because mesenteric lymph return is a vital contributor to intestinal bacteria-endotoxin translocation, these adverse effects further lead to uncontrolled inflammation, vascular hyperpermeability and hyporeactivity, immunosuppression, and subsequent multiple organ injury. Thus, increasing lymph formation and transport via regulation of lymphatic function may serve as a means of antagonizing the pathogenesis of T/HS.

Keywords

Trauma-hemorrhagic shock · Lymphatic function · Mesenteric lymph

5.1 General

The microcirculation refers to the circulation of blood, lymph, and interstitial fluid that communicates substance, informatory factors, and energy between tissues and cells, including blood microcirculation, lymphatic microcirculation, and tissue

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channels, which is essential to many functions of the organism. Asellius conducted the first documented study of the lymphatic system in the 1600s. Since then, biologists have investigated different aspects of the lymphatic system and developed a keen scientific interest in lymphatic biology. This interest has led to advances in the comprehension of lymphatic system, particularly in regard to lymphatic angiogenesis and function, lymph formation, and transport.

In general, the main pathway of interstitial fluid return to blood circulation is through the absorption of initial lymphatic to form lymph, as well as by venules. The initial lymphatics have discontinuous endothelial cells, and the fenestra and gap of initial lymphatic endothelial cells are larger, which contribute to the absorption of interstitial fluid, macromolecules, particulates, and immune cells derived from the corresponding tissue into lymphatic spaces to form lymph. Afterward, the lymph flows into initial lymphatics, micro-collecting lymphatics, collecting lymphatics, lymph trunk, and ultimately through thoracic duct or right lymphatic duct, goes into blood circulation, which is the so-called lymph circulation. In physiological state, the total quantity of lymph formed in 1 day is normally 2–4 L, which eventually returns to the blood through the lymphatic system. Thus, lymphatic system plays an important role in body fluid regulation, material absorption, lymphocyte recirculation, and immune modulation. Lymphatic contractile motility is a major propellant power for the lymph circulation. The disturbance of lymphatic motility contributes to the pathogenesis of lymph circulation disturbances-related disease, such as lymphedema [1], inflammatory bowel disease [2], metabolic syndrome [3], etc.

A variety of severe trauma such as military injury, traffic accidents, natural disaster, major operation, and postpartum massive hemorrhage severely lead to the loss of blood volume and tissue hypoperfusion and ultimately the occurrence of trauma-hemorrhagic shock (T/HS) manifested by acute circulatory disturbance, severe tissue hypoxia, and causing grave consequences of cell or tissue injury and function disturbance [4]. After 200 years of research and practice in trauma field, it has been established that intractable T/HS involves in uncontrolled inflammatory response [5], vascular hyporeactivity [6], vascular hyperpermeability [7], myocardial dysfunction [8], energy metabolic disorder [9], blood coagulation dysfunction [10, 11], and immunosuppressive [12]. Although specific therapy targeting corresponding mechanism has promoted the progress of prophylaxis and treatment of T/HS, the fatality rate remains high. Between 2000 and 2010, traumatic injuries have been reported to become the leading cause of death in the United States under 46-year-old peoples, and blood loss accounts for 39% of all traumatic deaths [13]. Therefore, it is urgent to extend the studies focusing on the pathogenesis of severe T/HS so that shock-related mortality can be reduced.

In recent years, researchers have focused on the role of the lymphatic system in the development of T/HS. They found that favorable lymphatic contractibility enhanced the function of lymph formation and transport in the early stage of T/HS, which play an important compensatory role in the improvement of shock. Conversely, the reduced lymphatic function of lymph transports due to lymphatic hypocontractibility leads to tissue edema and refractory shock. Moreover, mesenteric

lymph return is also a vital contributor to intestinal bacteria-endotoxin translocation, which further results in remote organ injury and irreversible shock. Therefore, targeting the regulation of lymphatic contractibility, lymph formation, and transport may ameliorate the prevention and treatment of severe shock. Hence, in the current article, we review the role of lymph formation and transport in the pathogenesis of T/HS and provide new information on the prevention and treatment of severe hemorrhagic shock.

5.2 Role of Lymphatic Function in the Development of T/HS

It is generally recognized that the natural progress of T/HS includes two phases. The first phase manifested by hypotension is induced by trauma or hemorrhage. In this phase, the loss of effective circulatory blood volume triggers the activation of sympathetic-adrenal medullary system and renin-angiotensin system, by which vasoconstrictive agents are released into systemic circulation. Microvascular inconsistent contraction of skin or mucosa, skeletal muscle, and abdominal viscera causes a redistribution of blood flow and ensures adequate blood supply to the heart and brain. This process has an important compensatory effect. The next phase is a rescue process mainly by aggressive fluid resuscitation. The aggressive fluid resuscitation can correct microcirculatory ischemia and restore the levels of blood pressure. However, not all of patients equally benefit from the effective treatment at the golden hour, and some peoples still develop into refractory hypotension and even death because of a variety of complicated factors [14].

It has been reported that, from 2001 to 2011, over 80% of US battlefield deaths that should be potentially survivable were due to severe hemorrhage [15]. The mechanism underlying death from severe hemorrhage is that blood perfusion of organs was not restored timely, which results in the subsequent vasomotor dysfunction including vascular hyporeactivity and paralytic vasodilatation, as well as vascular endothelium hypoxic injury causing vascular permeability. This adverse effect leads to interstitial fluid silted up and tissue edema. Therefore, the key of T/HS cure is to restore the blood perfusion of organs and vascular tone timely, thereby promoting the return of interstitial fluid to vascular compartment that increases the circulating blood volume. With the restore of blood perfusion, tissue edema and ischemic hypoxia will dissipate quickly so that the tissue can maintain a primary blood perfusion under hypotension conditions. Hence, the return of interstitial fluid into blood circulation is necessarily required in the treatment of T/HS. It is generally accepted that the factors that increase the formation of interstitial fluid and decrease the return to blood circulation include increased capillary hydrostatic pressure, decreased plasma colloid osmotic pressure, and vascular hyperpermeability. Besides these factors, lymphatic system dysfunction for the interstitial fluid absorption and lymph transportation is also a key contributor. Therefore, improvement of the lymphatic system function in different phases of severe T/HS is another strategy with great significance to treat T/HS.

5.2.1 Changes of Lymphatic Contractibility During T/HS

The observation of lymphatic microcirculation in vivo indicates that contraction frequency of mesenteric lymphatics is approximately 6–8/min before hemorrhage. When mean blood pressure (MAP) declines to 80 mmHg induced by blood bleeding, lymphatic spontaneous contraction frequency and three contractility indices [including fractional contraction index (Index I), which is the lymphatic contractile amplitude and positively correlated with the lymph volume transported during a single contraction; total contractile activity index (Index II), which reflects the lymphatic contractile ability in units of time; and lymphatic dynamic index (LD-Index), which reflects the lymph transport situation as a whole] are maintaining at normal or slightly higher levels, suggesting that lymph return is not deteriorated at this stage and plays compensatory and anti-shock roles at early stage of shock, combining autotransfusion, auto-fluid transfusion, and blood redistribution. When MAP declined to 40 mmHg by hemorrhage, with extending hypotension, contraction frequency of mesenteric lymphatics is decreased significantly, approximately 1–2/min, and three contractile indices also are reduced significantly. After fluid resuscitation, along with the improvement of blood microcirculation, the contraction frequency of mesenteric lymphatic is restored, while lymphatic contractility indices are also enhanced. However, along with the progression of shock, mesenteric lymphatic contraction frequency and contractile indices were again decreased in the beginning of 2 h after fluid resuscitation [16]. In addition, Johnston et al. reported that hemorrhagic shock induced a decrease in lymph volume [17]. Higaki et al. found that both contraction frequency and amplitude of the mesenteric lymphatics were reduced, along with the increase of blood loss in rats following hemorrhagic shock [18].

In order to observe the changes of lymphatic contractibility following T/HS with the absences of neural and humoral factors, we established isolated lymphatic contractile observation technology using the equipment of "microvascular perfusion system," and evaluated the lymphatic contraction function using the indices of contractile frequency (CF), tonic index (TI), contractile amplitude (CA), and fractional pump flow (FPF). The results showed that with the sustaining hypotension, under different transmural pressure, lymphatic contraction performance had a bi-phase change, exhibiting enhanced changes at 0 h and 0.5 h of post-shock and decreased changes at 2 h and 3 h of hypotension [19]. Moreover, at 3 h after hemorrhagic shock with resuscitation, the rat-isolated lymphatic CF, FPF, and TI were significantly decreased more than that of the sham rats [20].

These studies confirmed the phenomenon of lymphatic hypo-contractibility following severe T/HS in vivo or in vitro, which is involved in the pathogenesis of T/HS.

5.2.2 Changes of Lymphatic Reactivity During T/HS

Lymphatic constriction is not only concerned with lymphatic smooth muscle cells (LSMCs) but also modulated by humoral factors, such as adrenalin, acetylcholine, nitric oxide (NO), 5-hydroxytryptamine, endothelin, histamine, and so on. We

denominated the lymph contractility vessels to vasoactive substances as lymphatic reactivity according to the concept of vascular reactivity.

The observation of lymphatic microcirculation in vivo indicates that the static caliber of rat mesenteric lymphatic microvessels can be constricted by noradrenalin (NE) dosage dependently. But at the late stage of shock, the reactivity of lymphatic vessels to NE is reduced remarkably. The concentration-response curve of lymphatic vessels to NE markedly shifts to the left, and the value of EC50 increases from 4.8×10^{-9} mol/L to 10^{-4} mol/L [16]. In further experiments, the intralymphatic pressor response to intravenous administration of NE (5 μg/kg) through the femoral vein began to wear off from 1 h of hypotension in T/HS rats. The difference values of F, Index I, Index II and LD-Index before and after administration of NE were used as indices to assess the lymphatic reactivity *in vivo*. And the results showed that the value of lymphatic △F, △Index II, and △LD-Index at 1 h of shock and the value of △F, △Index I, △Index II, △LD-Index at 1.5 h and 2 h of shock were significantly lower than that of the sham group. Moreover, the value of lymphatic △F, △Index I, △Index II, and △LD-Index at the above three times of post-shock was significantly lower than that of pre-shock conditions [21].

The contractile response of isolated lymphatic segments to NE was measured by using a pressure myograph. The difference of contractile frequency was used to assess the lymphatic reactivity. Results indicate that contractile response to gradient concentration of NE and Ca^{2+} of lymphatics isolated from rats with maintained hypotension 2 h was significantly decreased. The reactivity of these lymphatics to NE and Ca^{2+} was significantly increased following incubation with the calcium sensitizer Ang II; the reactivity of these lymphatics was significantly decreased after incubation with the calcium sensitivity inhibitor insulin [21]. The results suggest that lymphatic hyporeactivity after shock is relevant to the decreased calcium sensitivity.

In next set of experiments, the response of isolated lymphatic vessels to substance P (SP) was measured by using pressure myograph system. The difference value (△CF, △TI, △CA, and △FPF) of CF, TI, CA, and FPF before and after administration of SP was used as indices to assess the lymphatic reactivity. The results indicate that the lymphatic reactivity presented a biphasic change in the hypotension-sustained process, exhibiting an increase at 0.5 h of shock and decline at 2 h and 3 h of shock [22]. The study also found that the contractile response of lymphatic vessels isolated from 3 h at the end of fluid resuscitation following hemorrhagic shock rats to SP showed a significant downward trend [20].

These results in vivo and ex vivo indicate that lymphatic reactivity is characterized by a biphasic change, which increases in early phase and declines in the later stage of T/HS. The high consistency of lymphatic reactivity and contractibility indicates that the decreased lymphatic vessels contractile reactivity to vasoactive substances is one of the important mechanisms underlying lymphatic hypo-contractibility indirectly.

In summary, in the early phase of hemorrhagic shock, the increased lymphatic contractibility and reactivity promotes the return of interstitial fluid to blood circulation via lymphatic system. The return of interstitial fluid to blood circulation

combined with other changes, such as autotransfusion, auto-infusion, blood flow redistribution, and so on, plays together to build a compensatory defense line in early stage of hemorrhagic shock. With the prolonging of hypotension and persistent impingement of reperfusion, the reduced power of lymphatic drainage due to the suppression of lymphatic reactivity and contractility and the damage of lymphatic endothelium ultrastructure is one of the most important contributors to tissue edema, which aggravates circulatory collapse and irreversible shock. Therefore, how to control the lymphatic contractile function to promote the return of interstitial fluid to blood circulation via lymphatic system is especially important to compensate the damage of shock. These results suggest that, besides blood microcirculation disorders, lymph microcirculation dysfunction is involved in the development of T/HS and is a critical mechanism for shock transition from compensation to decompensating condition. It is concluded that lymph microcirculation dysfunction is a vital component of microcirculatory impairment theory of shock pathogenesis.

5.3 Role of Posthemorrhagic Shock Mesenteric Lymph in the Pathogenesis of Irreversible Shock

Trauma and acute hemorrhage activate the sympathetic-adrenal medulla system and increase the concentrations of norepinephrine (NE) and epinephrine in peripheral blood, along with the activation of renin-angiotensin system (RAS), thereby inducing the redistribution of blood and the increase in interstitial fluid return. As a result, the skin, skeletal muscle, and abdominal organs, including the intestine, spleen, liver, kidney, etc., became the prior ischemic organs following T/HS. Of which, the intestine is a primary organ susceptible to low perfusion and ischemic injury. With the continuation of hypotension, the gradually amplified ischemic injury and inappropriate fluid resuscitation-induced reperfusion injury increase intestinal mucosal permeability and cause intestinal barrier dysfunction, which leads to gut-derived bacteria and endotoxin translocation and enterogenous infection. Subsequently, the abovementioned factors further cause and aggravate the damage of distant organs [23]. Therefore, gut is considered as an important portal of entry for bacteria that plays a critical role in trauma or hemorrhage-induced multiple organ dysfunction syndrome (MODS). Moreover, intestinal injury also becomes a key link of T/HS developing to sepsis [24–27].

It has been identified that rich lymphatics and lymphatic system are present in intestinal tissue. A large amount of lymphatic capillary network is distributed in the mucosa, muscularis, and serosa of small intestine. The lymphatic capillary vessel in the mucosa derives from central lacteals in the lamina propria of the intestinal villus and then converges into the mucosa lymphatic capillary network, which inosculates with lymphatic located in the submucosa to form lymphatic plexus. Open junction is the gap between two lymphatic endothelial cells, which width is >30 nm. Open junction in different organs displays different functional states, and its average open rate is 1–6% approximately. It has been reported that the hollow gastrointestinal tract has more open junctions [28]. In resting state, the gap width of open junction

is about 30–120 nm, usually, which is the histological foundation of proteins, tracers, macromolecules, and cells transfer into the intralymphatic compartment. But, the open rate of open junctions can reach 50% in mild injury conditions of lymphatic endothelial cells [29].

In view of mesenteric lymphatic feature that is rich of open connections and can absorb macromolecules, together with intestinal barrier failure as well as lymphatic endothelial barrier degeneration induced by sustained hypoxia in the late phase of shock, it is possible that the endotoxin, bacteria, and other bioactive substances return to blood circulation through mesenteric lymphatic pathway. Since the 1990s, Moore, Deitch et al. had turned attention to the role of mesenteric lymph playing in acute lung injury (ALI), myocardial contractile dysfunction, and inflammatory cascade induced by T/HS. They concluded that mesenteric lymph is the key link of T/HS leading to organ injury and sepsis [26, 30–34]. In recent two decades, posthemorrhagic shock mesenteric lymph (PHSML) involved in the pathogenesis of intractable shock from many aspects such as vascular hyporeactivity and hyperpermeability, immunosuppressive, and so on have been established.

5.3.1 Role of PHSML in T/HS-Induced Organ Injury

T/HS-induced major organ dysfunction and injury play a triggering role into the development of irreversible shock, which is a leading cause for death. Recently, many scholars investigated the mechanisms of PHSML return in T/HS-induced organ damage, and their results shed light on the prevention and treatment of organ injury after T/HS.

5.3.1.1 Role of PHSML in T/HS-Induced ALI

Following T/HS, the pulmonary circulation blood volume is decreased correspondingly with T/HS-decreased systemic circulation blood volume. The abnormal hemodynamics leads to ALI, combining with uncontrolled inflammatory response and gut-derived bacteria-endotoxin translocation. ALI is the most likely and the first major organ injury after T/HS, which is the main cause for death in T/HS patients. Therefore, it is of importance to develop new therapeutic strategies for the prevention of ALI and cure of severe T/HS.

In 1998, Magnotti et al. [35] firstly reported that the mesenteric lymph division before hemorrhagic shock prevented shock-induced pulmonary hyperpermeability to Evans blue dye and increases in alveolar apoptosis and pulmonary myeloperoxidase (MPO) levels, while mesenteric lymph obtained from rats of hemorrhagic shock with resuscitation increased human umbilical vein endothelial cells (HUVECs) monolayer permeability to rhodamine (10 K) in vitro, but not post-shock portal blood plasma. The data indicate that gut-derived factors carried in the mesenteric lymph rather than the portal circulation are involved in the pathogenesis of shock-induced ALI. Deitch et al. [36] further showed that mesenteric lymph division prevented hemorrhagic shock-induced lung hyperpermeability at 3, 6, 12, or 24 h post-shock, PHSML samples with concentrations of 1 or 10% all increased the

HUVEC permeability, and the greatest relative increase in HUVEC permeability was observed in the 3- and 6-h post-shock samples. Sambol et al. [37] found that mesenteric lymph duct ligation 7 days prior hemorrhagic shock continued to prevent shock-induced lung injury, exhibiting lower concentrations in Evans Blue dye, bronchoalveolar fluid (BALF) protein, and pulmonary MPO activity; these data also suggest that the role of mesenteric lymphatic duct ligation in alleviating shock-induced lung injury continues at least 1 week. Furthermore, Gonzalez et al. [38] found that mesenteric lymphatic diversion abrogated the effect of hemorrhagic shock, increasing surface expression of polymorphonuclear neutrophils (PMNs) CD11b and MPO activity in pulmonary tissue, and reduced lung PMNs accumulation. Moreover, mesenteric lymph duct ligation prevented T/HS-induced increased P-selectin and intercellular adhesion molecule-1 (ICAM-1) expression in pulmonary tissue at 3-h and 24-h of post-shock [39].

In in vitro experiments, PHSML induced activation of human PMNs, primed PMNs for superoxide production, as well as increased surface expression of CD11b [40]. PHSML also activates human pulmonary microvascular endothelial cells (PMVECs) for increased ICAM-1 expression and stimulates PMNs to adhere to endothelial cell monolayers adherence, resulting in PMVECs death [41]. These data indicated PHSML promotes PMNs adherence to vascular endothelial cells and induces PMN-mediated endothelial cell injury. Furthermore, PHSML incubation at a 10% concentration, but not traumatic-sham shock or post-T/HS portal vein plasma, inhibited the activities of HUVECs and rat PMVECs, resulted in more than 95% mortality rate of the HUVECs and rat PMVECs after a 16-h incubation, and increased the permeability of both HUVEC and rat PMVEC monolayers [35, 42].

There is evidence showing that PHSML treatment caused the appearance of the classic morphologic signs of apoptosis, including membrane blebbing, cell shrinkage, and apoptotic body formation, and a DNA laddering pattern was also observed [43, 44]. Meanwhile, PHSML incubation increased signs of apoptosis, such as caspase-8, caspase-9, and caspase-3 in HUVECs; however, the broad-spectrum caspase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp (zVAD) did not fully prevent PHSML-induced HUVECs cell death, which suggested the mechanism of PHSML-induced cell apoptosis involves both caspase-dependent and caspase-independent pathways [43]. Barlos et al. [45] found that PHSML treatment decreased the viabilities of HUVECs and human PMVECs and induced the nuclear translocation of apoptosis-inducing factor (AIF); AIF silencing in HUVECs reversed the PHSML-induced cytotoxic effects of cell viability and DNA fragmentation. PHSML activated caspase-3-mediated apoptosis in human alveolar type II epithelial cell (A549) lines, which was partially abrogated by zVAD. However, PHSML did not cause the nuclear translocation of AIF in A549 cells. In conclusion, PHSML-induced PMVECs apoptosis occurs through an AIF-dependent caspase-independent pathway, whereas epithelial cell apoptosis is by a caspase-dependent pathway. The result from our lab showed that the apoptosis rate of PMVECs was 9.86 ± 3.24% after exposed to PHSML at a final concentration of 4% for 4 h which was significantly higher than that of other groups. At the same time, the mRNA expressions of fas, fas L, and bax were higher, along with

a lower mRNA expression of bcl-2, compared to the control group. These data demonstrated that the mechanism of PHSML-inducing apoptosis of rat PMVECs is related to the enhanced expression of apoptosis trigger genes fas, fas L, and bax and depressed expression of apoptosis inhibiting gene bcl-2.

Senthil et al. documented that, although no hemodynamic changes was detected during the whole experiments procedure, intravenous infusion of PHSML increased lung permeability, MPO activities (marker of neutrophil sequestration), inducible nitric oxide synthase (iNOS), and NO levels [46]. Furthermore, the histologic appearance of rats received intravenous infusion of PHSML characterized by interstitial edema, inflammatory cell infiltration, and alveolar hemorrhage [46]. In a similar experiment conducted by Wohlauer et al. [47], cross-transfusion of PHSML into a normal rat in real time provoked acute lung injury-like changes, such as PMNs accumulation, increased protein level in BALF, and vascular hyperpermeability.

Recent studies have identified that besides through pathogen-associated molecular patterns recognizing microbial components, TLR4 can receive endogenous dangerous signaling molecules released by stress or injured cells via damage-associated molecular patterns [48]. It has been confirmed that the TLR4 signaling pathway has a central role in the pathogenesis organ injury after T/HS [5]. Reino et al. reported that compared with wildtype mice, porcine PHSML did not induce lung injury of TLR4mut mice. Similarly, TLR4 downstream signaling adapters TRIF and Myd88 deficiency fully and partially attenuated PHSML-induced lung injury, respectively. Moreover, the endotoxin removal PHSML did not relieve its lung injurious effects [49]. These results suggest that TLR4 can respond to sterile endogenous dangerous molecules and trigger the autoimmune inflammatory response without bacterial invasion. Therefore, as endogenous dangerous signaling molecules, injurious proteins and lipids contained in PHSML cause the tissue injury and the release of newly produced dangerous molecules, which further lead to the ALI ultimately.

5.3.1.2 Role of PHSML in T/HS-Induced Myocardial Injury

Clinical and experimental studies have shown myocardial contractile dysfunction plays an important role in maintaining blood flow dynamics and in ensuring tissue perfusion. Therefore, cardiac systolic dysfunction is a key link of aggravating microcirculation disorder and causing other organ injuries following severe T/HS, which is a major cause of death [50–52]. Hence, protection of cardiac structure and function is main target of prevention and cure of severe T/HS.

Previous studies showed that mesenteric lymphatic duct ligation alleviated myocardium injury, reduced the levels of free radical, pre-inflammatory factors of TNF-α and IL-6 in cardiac muscle tissue following hemorrhagic shock [53], induced down-expression in bax and up-expression in bcl-2, and, thereby, lessened the myocardial apoptosis. Furthermore, using a Langendorff-isolated heart preparation, the results showed that mesenteric lymphatic duct ligation significantly enhanced the left ventricular systolic pressure (LVSP), maximal rates of the left ventricular developed pressure rise and fall (±dP/dtmax), and response to increases in coronary flow rates and Ca^{2+}, thereby, improved the myocardial contractile function [54]. Similarly, PHSML drainage plays a protective effect on myocardial contractile function [55].

Perfusion of PHSML to hearts obtained from normal rats decreased the cardiac function indices of ±dP/dtmax and LVDP [55]. Acute application of PHSML induced dual inotropic effects and abnormal electrophysiological properties of cardiac myocytes [56]. Furthermore, at 24 h after intravenous infusion of PHSML, the isolated hearts have contractile dysfunction, exhibiting decreases in LVDP and ±dP/dtmax and a blunted inotropic response to Ca^{2+} [56].

These studies indicate that PHSML return directly induces myocardial dysfunction and negative inotropic effects on the myocardium; the mechanism is related to the suppression of calcium channel activity induced by PHSML. However, the particular mechanism by which T/HS-induced myocardial dysfunction should be further investigated.

5.3.1.3 Role of PHSML in T/HS-Induced Acute Kidney Injury

The kidney is one of the prior ischemic organs following T/HS and also is one of the prior damaged organs [57]. Based on the theory of microcirculation disturbance, fluid resuscitation is carried out extensively in various ways, and effectively recover blood perfusion of kidneys. Hence, the morbidity of acute kidney injury (AKI) induced by ischemia directly was significantly reduced [58]. However, due to the limitations of fluid resuscitation opportunity and means, and gut-originated bacterial endotoxin translocation, some patients appeared with ischemic injury induced by different levels of low perfusion, reperfusion injury induced by improper fluid resuscitation, endotoxin damage induced by bacterial endotoxin translocation, and refractory hypotension induced by vascular hyporeactivity, which consequently causes AKI or acute renal failure (ARF). Moreover, AKI or ARF can further result in the accumulation of metabolites and thus leads to a series of internal environment disorder (e.g., acidosis, hyperkalemia), which is one of the key link by which hemorrhagic shock induces organ injury and even MODS. So, AKI has become one of the most vital factors of irreversible shock [59]. Therefore, it is essential to investigate the possible mechanisms and therapeutic measures of AKI following T/HS.

The previous researches have shown that blockage of PHSML return following hemorrhagic shock with liquid resuscitation by mesenteric lymphatic duct ligation alleviated the renal tissue injury; reduced the levels of free radical, NO, tumor necrosis factor α (TNFα), and IL-6 in renal tissue; and reduced the activity of the MPO, which reflects the PMNs sequestration in the renal tissue [60]. The mesenteric lymphatic duct ligation decreased renal cellular structural damage after hemorrhagic shock, reduced the activation of 5-lipoxygenase (5-LO) and 5-lipoxygenase-activating protein (FLAP) in renal gap, and decreased the level of leukotriene in urine [61]. The study on continuous low perfusion after hemorrhage found that mesenteric lymph drainage from 1 to 3 h after hypotension reduced renal tissue damage, reduced the activity of trypsin, and the levels of lactic acid, ICAM-1 and advanced glycation end products in renal tissues [62]. Han et al. found that PHSML drainage reduced the renal tissue damage and decreased hydrogen sulfide, cystathionine-γ-lyase (CSE), and TNFα levels in renal tissues. Meanwhile, L-propargylglycine (PPG), an inhibitor of CSE, enhanced the effect of PHSML drainage. However, this beneficial effect of PPG was reversed by sodium hydrosulfide

hydrate administration [63]. These data indicated that the PHSML return is involved in the mechanism by which hemorrhagic shock induced AKI.

To further study the mechanism of PHSML return on the AKI after hemorrhagic shock, we used DNA microarrays and found that mesenteric lymphatic duct ligation results in differential expression of 34 known genes in renal tissues after hemorrhagic shock with resuscitation. The differential expressed genes encoding proteins function were involved in signal transduction, transcriptional regulation, metabolism, transportation, cell growth, cell cycle, cell adhesion, cell composition, and so on [64]. The determination of proteomics techniques found that PHSML drainage also decreased the expression of Atp5b and the actin subtype in renal tissues after hemorrhagic shock with resuscitation. These proteins functions are involved in energy metabolism, cytoskeleton, and movement [65]. These studies suggest that the mechanism of PHSML-induced renal injury after hemorrhagic shock is related to the abovementioned target. However, the exact mechanism needs to be further explored.

In conclusion, PHSML return is a key link of T/HS-induced multiple organ injury; the measures acting to reduce mesenteric lymph return under severe T/HS status provide new theoretical foundation for prevention and treatment of T/HS. However, seeking new intervention methods for more clinical applications are still the main direction in the future.

5.3.2 Role of PHSML in T/HS-Induced Vascular Hyporeactivity

Although part of severe shock patients received aggressive fluid resuscitation, blood pressure did not increase significantly, peripheral resistance vessels did not appear obvious response to vascular active substances, and hypotension did not have considerable improvement after administration of vasoactive drugs. This phenomenon indicated that low perfusion of organs does not ameliorate the aggressive fluid resuscitation and vascular hyporeactivity has been occurred [66]. A large number of documents reported that vascular hyporeactivity caused by trauma, sepsis, hemorrhage, etc. is one of the important pathogenesis underlying refractory hypotension, no-reflow phenomenon, organ hypoperfusion, and microcirculatory failure and is one of the reasons of refractory shock difficult to cure. Thus, correcting vascular hyporeactivity timely is beneficial to the reversal of shock [6, 67–69]. Therefore, to explore the mechanism of vascular hyporeactivity in severe shock and then to seek controlling measures play some positive significance for prevention and treatment of severe shock. The previous study showed that various factors, such as adrenergic receptor desensitization, membrane hyperpolarization of vascular smooth muscle cells (VSMCs) induced by K_{ATP} activation [70–74], calcium desensitization of contractile proteins, etc., involve in the development of vascular hyporeactivity [6]. Recent researches have shown that PHSML participated in the vascular hyporeactivity after severe T/HS.

It has been identified that blocking the return of PHSML into blood circulation at immediate shock can increase the pressor response of T/HS rats in vivo [75]. In

addition, blocking the return of PHSML into blood circulation can improve the contractile response of isolated vascular vessels from T/HS rats to a gradient concentration of NE and Ca^{2+} using a severe T/HS rat model by means of mesenteric lymph duct ligation and mesenteric lymph drainage [75]. Moreover, calcium sensitizer angiotensin II can enhance the contractile response of vascular vessels isolated from T/HS rats to NE and Ca^{2+}; calcium desensitizer insulin can suppress the protective effect of blocking PHSML return for the contractile response of isolated vascular vessels NE and Ca^{2+}. These results suggested that reducing the return of PHSML into blood circulation improves vascular reactivity through the increase of vascular calcium sensitivity [75]. Data from ex vivo experiments demonstrated that PHSML harvested at 1–3 h shock period decreases vascular reactivity of normal mesenteric artery rings, and PHSML harvested at 0–0.5 h shock period increases vascular reactivity; PHSML harvested at 0–0.5 h shock period has no influence for the mesenteric artery rings. In further in vivo experiments, the data indicated that vascular reactivity in blocking the return of PHSML at 1 h after shock is significantly higher than those in blocking the return of PHSML at immediate shock. It approaches that level of those in sham group. These data suggested that injurious effect of mesenteric lymph arises from 1 h after shock [75].

Studies have shown that the mechanism underlying T/HS inducing vascular hyporeactivity and calcium desensitization involves in the small GTP-binding proteins RhoA [76] and Rac1 [77], the balance between RhoA and Rac1 [78, 79], Rho-associated kinase (ROCK) [80–82], protein kinase C (PKC) [83–86] and its subtypes [83, 87, 88], protein kinase G (PKG) [83, 89], etc. The calcium sensitivity of VSMCs mainly depends on striking a balance between the activity of myosin light chain phosphatase (MLCP) and myosin light streptokinase (MLCK). Their combined effects determine the phosphorylation level of 20Kda myosin light chain (MLC20) and then change vascular calcium sensitivity and reactivity [90, 91]. The mechanism that RhoA, Rac1, ROCK, PKC, and PKG adjust vascular reactivity is realized by changing the phosphorylation level of MLC20 [78, 79].

In further experiments, results suggest that PHSML drainage increases the RhoA, p-RhoA, and p-MLCK levels of mesenteric artery harvested from T/HS rats significantly [92, 93] and mesenteric lymph duct ligation or PHSML drainage increases the activity of PKC and p-PKC [94] and decreases the content of PKG and p-PKG [95]. Further findings indicated that RhoA inhibitors C3 transferase and MLCK inhibitors ML-7 significantly suppress the protective effect of PHSML drainage for vascular reactivity and calcium sensitivity of shock rats, respectively [92, 93]; PKC inhibitors staurosporine and PKG agonist 8Br-cGMP significantly reduce, and PKC agonist PMA and PKG inhibitor KT5823 significantly enhance the protective effects of mesenteric lymph duct ligation or PHSML drainage for vascular calcium sensitivity [94, 95]. In addition, the ROCK inhibitor fasudil significantly decreases the protective effects of mesenteric lymph duct ligation or PHSML drainage for blood calcium sensitivity [96].

These above studies demonstrate that the return of PHSML into blood circulation is an important mechanism underlying vascular hyporeactivity after severe T/HS insult. Molecular targets of PHSML decreasing vascular reactivity involve in

Rho, ROCK, PKC, PKG, and MLCK signaling proteins. These results partly illustrate the mesenteric lymph mechanism underlying vascular hyporeactivity induced by severe T/HS insult. The present work considering PHSML as the breakthrough point provides the experimental basis for the intervention of T/HS vascular hyporeactivity. It provides a new insight into the prevention of severe shock by targeting lymph modulating vascular reactivity. Besides, recent studies showed that the mitochondrial dysfunction in VSMCs is involved in severe shock-induced vascular vasoreactivity, and mitochondrial protectors, such as CsA and polydatin, have a protection on the treatment of shock-induced hypotension [97, 98]. However, whether PHSML return-induced vascular hyporeactivity is related to the mitochondrial injury, it remains unclear.

5.3.3 Role of PHSML in T/HS-Induced Vascular Hyperpermeability

Previous studies have shown that vascular hyperpermeability is a key process of severe shock-induced capillary leak and systemic tissue edema, thereby causing cellular hypoxia and microcirculation disturbance. These adverse effects further induce refractory hypotension that develops MODS and multiple organ failure (MOF). Therefore, understanding the mechanisms underlying severe shock-induced vascular hyperpermeability would help develop an effective measurement for prevention and treatment of vascular hyperpermeability and severe hemorrhagic shock.

The study that the role of PHSML return into blood circulation plays in ALI after severe hemorrhagic shock found that both of mesenteric lymph duct ligation and PHSML drainage reduced pulmonary microvascular permeability and tissue edema after T/HS, which is indicated by increasing lung tissue dry/wet ratio, decreasing the permeability for Evans blue of respiratory membrane [35]. Besides the lung, PHSML drainage also reduced the permeability for Evans blue of the myocardium, liver, spleen, kidney, and intestine of T/HS rats and enhanced the tissue dry/wet ratio [99]. In vitro experiment, PHSML increased the permeability of endothelial monolayer derived from the femoral vein [100]. Meanwhile, PHSML infusion increased pulmonary microvascular permeability and pulmonary edema of normal rats. From the aspect of organ, these studies suggested that PHSML return into blood circulation is one of the important mechanisms underlying T/HS inducing vascular hyperpermeability; declining the return of PHSML may contribute to the alleviation of T/HS inducing vascular hyperpermeability.

It has been widely accepted that the integrity of vascular endothelial cells and cell-cell junctions is the structural foundation to maintaining the function of vascular endothelial barrier. In physiologic state, the permeability of vascular endothelial barrier is controlled by transcellular and paracellular mechanisms [101]. In general, transcellular pathway involving movement across cell membranes [102] is an important mechanism responsible for homeostasis of macromolecules between intra- and extravascular compartment such as albumin and lipid [103, 104]. Paracellular pathway describing movement through the space between adjacent cells is a critical

mechanism of governing endothelium permeability mediated by interendothelial junctions [105]. Transcellular transport is associated with the integrity and function of cytoskeletal proteins, while paracellular transport is controlled mainly by interendothelial junctions and its related molecules [105]. Therefore, the integrity of cytoskeleton and interendothelial junctions in vascular epithelial cells (VECs) is associated with the regulation of endothelial barrier function and vascular permeability.

The further study indicates that mesenteric lymph duct ligation reduced the apoptosis of endothelial as well as epithelial cells in the lung caused by T/HS [100], PHSML induced apoptosis of PMVECs cultured in vitro [44, 100], as well as the DNA ladder was observed in electrophoresis of cell nucleus DNA at same time [44]. Meanwhile, PHSML collected from T/HS rats caused morphological lesion and viability decline of HUVECs in vitro [99] and led to the decrease of transendothelial electrical resistance (TEER) and the increase of permeability for 40-kD FITC-albumin of HUVEC monolayer in vitro [106]. Moreover, PHSML also results in the downregulated expression of cytoskeletal protein F-actin and adherens junction protein VE-cadherin [106]. With primary cultured rat thoracic aortic vascular endothelial cells (TAVACs), we further confirmed that PHSML caused TAVACs injury, increased TAVACs monolayer permeability, and reduced expression of F-actin and VE-cadherin. We also got the same results from PMVECs treated with PHSML for 6 h. Monolayer permeability was increased and protein expressions of F-actin, VE-cadherin, ZO-1, and Claudin-1 was reduced, as well as increased expressions of ASK1 and p38 MAPK phosphorylation proteins. TRX1 (ASK1 inhibitor) or SB203580 (p38 MAPK inhibitor) treatment abrogated the adverse effects of PHSML on PMVECs, which may be due to the inhibition of ASK1 and p38 MAPK phosphorylation protein expression (unpublished data).

The above results showed that PHSML return is a fundamental mechanism of severe T/HS causing vascular hyperpermeability. The mechanism is related to PHSML-induced damage in the structure of vascular endothelial cells and inhibition in the expressions of intercellular adhesion proteins and endothelial cytoskeletal proteins, by which through the transcellular and paracellular pathways. Moreover, the ASK1/p38 MAPK signaling pathway is involved in the process of PHSML-induced vascular hyperpermeability.

5.3.4 Role of PHSML in T/HS-Induced Immunosuppression

Several studies indicate that immune depression after major surgery and T/HS has been implicated as an important factor in the pathogenesis of subsequent sepsis, bacteremia, and multiple organ failure [107]. Many studies have implicated immune-cell apoptosis, including splenocytes, NK cells, Peyer's patch T cells, polymorphonuclear cells, etc., as an important factor in the evolution of this post T/HS immune-suppressed state [108–112]. Due to mesenteric lymphatic pathway which is a major route of gut-derived factors translocation, the role of PHSML in the process of immunosuppression after T/HS is concerned in the field of critical medicine.

The recent studies have demonstrated that T/HS caused both thymic and splenic immune-cell apoptosis and that this increase in apoptosis was totally abrogated by mesenteric lymph duct ligation [113] or PHSML drainage [114, 115]. Meanwhile, PHSML drainage recovered the distribution of T-lymphocyte subgroup and the ratio of IFN-γ/IL-4 in peripheral blood, reversed T/HS-induced bax up-expression and bcl-2 down-expression in both thymic and splenic tissues, increased the proliferation index, and decreased p53 expression of thymocytes and splenocytes [114, 115]. PHSML injection caused splenic apoptosis in the WT mice, but not the TLR4mut mice; moreover, T/HS induced splenic apoptosis in the WT mice, but not the TLR4mut mice. The data confirmed that the process of gut-derived factors via PHSML leading to splenic and thymic immune-cell apoptosis is TLR4-dependent [113]. In addition, T/HS led to reductions of proliferation and cytokine production capacity of $CD4^+$ T lymphocytes and DCs, which were reversed by PHSML drainage. Moreover, the $CD4^+$ T lymphocytes and DCs isolated from normal mice were treated with PHSML collected from mice subjected to T/HS for different times. And the results showed that PHSML treatment had bi-phase effects on the proliferation and cytokine production capacity of $CD4^+$ T lymphocytes and DCs, exhibiting an enhanced effect at early stages and an inhibitory effect at later stages (unpublished data). These results indicated that PHSML return is involved in the process of T/HS-induced immune function disorder.

However, the detailed mechanism of immune dysfunction caused by PHSML needs be investigated in the future. Firstly, the exact mechanism of immune cells and inflammatory factors transfer through PHSML remains unclear. Secondly, it is also unclear that the component of PHSML involved in the regulation of immune cells after T/HS. In addition, the major target of PHSML activating immune cells is not clear. Thus, we should further focus on the role of PHSML in the process of immune dysfunction induced by T/HS, and provide the new theoretical foundation for prevention and treatment of critical patients through regulating the lymph formation and transport.

5.3.5 Components Analysis in PHSML

The above studies have demonstrated that PHSML return involves in the pathogenesis of T/HS and represents a leading cause of morbidity and mortality. Biologically active factors produced due to the intestine injury and transported by the mesenteric lymph are now thought to contribute significantly to the development of distant organ failure following T/HS. Therefore, many scholars determined the biologically active molecules in PHSML that lead to shock deterioration using multiple experimental methods, for the potential therapeutic interventions of T/HS targeting PHSML.

Adams et al. [116] found that there is a factor larger than 100kd in PHSML, which is toxic and fatal to the endothelial cells during severe hemorrhagic shock. Kaiser et al. [117] fractionated mesenteric lymph from T/HS rats and sham shock rats by solid-phase extraction (SPE) and ion-exchange chromatography (IEX) and

found that both of the two fractions fractionated by SPE or IEX had major detectable toxicity to endothelial cells from T/HS rats, whereas no toxicity was detected from sham lymph separations. Subsequent analysis of each SPE toxic fraction by gel electrophoresis and mass spectrometry suggested the toxicity was associated with a modified form of rat serum albumin (mod-RSA) and multiple lipid-based factors. Furthermore, Kaiser et al. mentioned that a single cationic peptide band was significantly increased in PHSML, but not in lymph from control animals subjected to trauma without hemorrhage. This peptide was subsequently identified as the N-terminal 24 amino acids of rat serum albumin (RSA) by mass spectrometry and amino acid sequencing. This albumin polypeptide induced the endothelial cell toxicity. It is therefore proposed that the significant increase in the albumin polypeptide is a marker for PHSML-induced endothelial cell toxicity [118].

With the development of proteomics technology, the differential proteins between PHSML and normal mesenteric lymph were investigated. Jordan et al. [119] found that gelsolin concentration was decreased in mesenteric lymph following hemorrhagic shock using the 2D-gels method and identification with mass spectrometry (MS-MS) that is possibly due to consumption by the actin scavenging system. Using the differential in-gel electrophoresis (DIGE)-based proteomics, Peltz et al. [120] found that there were 55 differential expressions proteins in PHSML, which were related to tissue injury, coagulation factors depletion, hemolysis, depletion of protective protease inhibitors, and an increase in abundance of lipid carriers. Fang et al. [121] investigated changes in proteome profiles between pre-shock and 3-h post-shock mesenteric lymph samples and found and confirmed four upregulated proteins, including serum albumin precursor, two isoforms of cytoplasmic actin, complement C3 precursor, and major urinary protein precursor, and downregulated haptoglobin in the PHSML samples. These altered proteins are functionally implicated in tissue inflammation. Zurawel et al. found that there were 127 altered proteins between PHSML and normal mesenteric lymph used in a rat model of hemorrhagic shock. In them, 74 proteins significantly decreased, along with 53 proteins significantly increased in the posthemorrhagic shock state, which were related to the loss of antiproteases, tissue injury, and coagulation disorder. Using a bioinformatics approach, Mittal et al. [122] reported the interaction of different proteins in PHSML, including 14–3-3 zeta, 14–3-3 epsilon, actin, aldolase A, calmodulin, cofilin 1, cystatin C, fatty acid-binding protein 4, profilin 1, prolyl 4-hydrolase, peptidylprolyl isomerase, and transgelin. Furthermore, D'Alessandro et al. [123] applied a label-free proteomics approach and found that the altered proteins in PHSML is related to coagulation dysfunction, uncontrolled inflammatory response, metabolic deregulation, proteases/antiproteases homeostasis disorder. Recently, Dzieciatkowska et al. [124] collected mesenteric lymph and plasma from critically ill or injured patients; after proteomic analyses, they found that there were 91 altered proteins between mesenteric lymph and plasma, which were related to coagulation disorder, cell lysis, inflammatory responses, immune function regulation, extracellular matrix remodeling, vascular hyporeactivity/neoangiogenesis, and energy/redox metabolic adaptation. These results revealed that there were altered proteins in PHSML, which may participate in T/HS. However, the pathophysiology of altered proteins is still unclear.

Besides the altered proteins in PHSML, scholars have paid attention to the lipid components of PHSML in the pathogenesis of T/HS. In 2000, the study from Gonzalez et al. [125] documented that both PHSML and PHSML lipid extracts inhibited human PMNs apoptosis and primed the NADPH oxidase, which were abolished by heat treatment or polymyxin B incubation. Moreover, isolated neutral lipids of PHSML primed superoxide production and respiratory burst of PMNs, phospholipase A2 (PLA2) inhibition before hemorrhagic shock abrogated PHSML-induced PMNs priming effects through reduction of the accumulation of neutral lipids [126], and PHSML after heat denaturing did not reduce PMNs priming [127]. These data implicate the lipid components of PHSML play a central role in the process of T/HS-induced activation of PMNs, which is related to gut PLA2.

Using the liquid chromatography/electrospray ionization mass spectrometry, Morishita et al. [128] identified the lipid mediators in PHSML and plasma and found that linoleoyl, arachidonoyl, and docosahexaenoyl significantly increased in the PHSML and linoleoyl and arachidonoyl induced the PMNs priming activity and the elastase release. Moreover, PHSML manifested cytotoxicity for HUVECs, which is associated with increased free fatty acids (FFAs), especially the FFA-to-protein ratio [129]. After treatment with lipase, the sham shock lymph also induced cytotoxicity similar to PHSML [129]. Using the gas chromatography, the main components of FFAs included palmitic, stearic, oleic, and linoleic acids, which induced cytotoxicity following incubation with HUVECs [129]. These data suggests that PHSML contains biologically active lipids which may be involved in the pathogenesis of T/HS-induced organ injury and inflammation.

In conclusion, PHSML contains a lot of biologically active components, including proteins of promoting proteolysis and oxidative stress and pro-inflammatory lipids, etc., turn into blood circulation, and initiates cascade reaction of systemic inflammation and organ injury; thereby the hemodynamic change evolved into multiple organ and system injuries. The development of proteomics, bioinformatics, and lipidomics provides a more comprehensive understanding of the compositions of PHSML. However, up to now, the detail compositions of PHSML are far from being clarified.

Based on the above series of studies, it has been recognized that sustained ischemic insult and subsequent reperfusion injury inducing gut barrier impairment as well as lymphatic endothelial barrier damage are the pathogenesis underlying the gut-derived infection; the mesenteric lymphatic duct is a critical way of gut-originated bacterial/endotoxin translocation besides portal vein by which injurious factors carried in the lymph avoids hepatic detoxification effect and enters the blood circulation directly along the thoracic duct and postcava vein. Mesenteric lymph back to blood circulation is one of the pivotal pathogenesis mechanisms underlying organ injury, uncontrolled inflammatory response, vascular hyperpermeability and hyporeactivity, immune dysfunction, and the subsequent MODS or MOF. Although a number of organ injurious substances carried in PHSML have been identified, these studies need to be improved, and further studies should be done through targeting active factors and toxic substances contained in PHSML to intervene T/HS. However, it is a long and hard way.

5.4 Regulatory Mechanism of Lymphatic Contractibility Following T/HS

Previous studies showed that the lymphatic contractility exhibits a biphasic change in the process of T/HS. In the early stage of shock, lymphatic contractility and reactivity are enhanced and contribute the absorption and transport of interstitial fluid to blood circulation, thereby playing an active role in compensation. In the late stage of shock, lymphatic contractility and reactivity are decreased, thereby deteriorating circulatory failure. Meanwhile, intestinal lymph pathway is the key way in intestinal injury inducing gut-derived infection leading to MODS or MOF. Therefore, targeting as lymph formation and transport, scholars concerned the regulation mechanism of lymphatic contractile function for the measurements of prevention and treatment of severe shock.

It has confirmed that lymphatic exhibits phasic and tonic contractile activity which is the power of lymph formation and transport. The various factors, such as nerve, humoral factors, local pressure gradient, and the homeostasis, influence the lymphatic contractility. Contractile protein and biological electric activities in LSMCs also are tightly related with lymphatic contractility. During T/HS, hypoxic- or ischemic-induced LSMCs injury and energy deficit caused lymphatic contractile dysfunction. In addition, changes of humoral factors and contractile protein in LSMCs are also involved in the regulation of lymphatic contractility and reactivity.

5.4.1 Regulatory Effects of SP and MLCK on Lymphatic Contractibility Following T/HS

Substance P (SP) is a neuropeptide associated with sensory innervation in lymphatic tissue and a modulator of lymphatics. Studies have demonstrated that SP (10 nM) could enhance the lymphatic chronotropy and contractility under the isometric condition. Gradually increasing the isometric preload could approximately make the contractive amplitude increase 1.6 times and the frequency increase 1.7 times at the absence of SP. However, SP treatment increased 1.9 times of contractive amplitude and 2.4 times of frequency. Under isobaric condition, the contraction amplitude could decrease by 0.6 times, and the frequency could increase by 1.8 times if the pressure elevated from 0.5 to 10 cmH_2O at the absence of SP. These results presented that SP treatment causes a slow increase in contractive amplitude and a fleet increase in frequency, and regulates the relationship among amplitude, frequency and pressure. Therefore, SP has positive inotropic and chronotropic effects on the lymphatic muscle, improving the contractile function of the lymphatic [130]. Therefore, SP was used as a tool for observing the reactivity of the lymphatic vessels in our study due to the role of SP in improving the lymphatic contraction.

Nepiyushchikh [131] demonstrated that SP treatment increased the lymphatic tonic contractions and phasic contraction frequency, but did not significantly alter the phasic contraction amplitude. SP administration activates MLCK, subsequently

increased the phosphorylated level of MLC20 in lymphatic muscle, and thereby enhanced the lymphatic tonic contractions. The selective MLCK inhibitor (ML-7) pretreatment of the lymphatics significantly decreased the SP-induced tonic contractions, increased the lymphatic diameter, and reduced the phosphorylation of MLC20. ML-7 combining with SP incubation to lymphatics also decreased the phosphorylation of MLC20. Neither ML-7 treatment alone nor combined with SP, the lymphatics phasic contraction amplitude did not significantly change. Hence, these data indicated that MLCK had little effect on the lymphatics phasic contraction amplitude. Due to SP treatment increasing the lymphatic contraction through MLCK, many scholars used the SP as MLCK an activator to observe the role of MLCK in modulation of the lymphatic contraction function.

In the studies of lymphatic contractile following hemorrhagic shock, we discovered that SP treatment could not change the phasic contraction amplitude but could enhance the phasic contraction frequency and the lymphatic tonic contractions, under multiple transmural pressures (1, 3 and 5 cmH_2O), thereby enhancing the lymphatic pump function of shock. The ML-7 caused significant decreases in the tonic contractions and frequency in the early stage of shock. Concurrently, ML-7 inhibited the SP upregulation effect on lymphatic contraction [132, 133]. These results revealed that MLCK is the key enzyme of regulating the lymphatic muscle contractibility and is involved in the regulation of lymphatic contraction in the progress of hemorrhagic shock. But the detail mechanism still needs to be further studied.

5.4.2 Regulatory Effect of NO on Lymphatic Contractibility Following T/HS

Under physiological condition, lymphatic endothelial cells only express endothelial nitric oxide synthase (eNOS), which depends on intracellular calcium ions. Lymphatic contraction causes lymph flowing and produces shear stress that further activates eNOS expression in the lymphatic endothelial cells for the production of NO, and lymphatic valves and capillary wall appear a rapid and transient increase in NO that induce lymphatic diastolic relaxation. The content of NO will decrease along with lymphatic diastole and induce the next contraction [134]. In the process of lymphatic contraction, the [NO] is increased in proportion to the contraction frequency; and the [NO] is decreased following contraction weakened [135]. These results showed there is an accompanying change of NO production during lymphatic periodic contraction and relaxation. The periodical change of NO in lymphatic segment participates in the regulation of lymphatic contraction, relaxation, and tension, contributes to maintain the lymph formation and fluctuation change of flowing volume and pressure in the process of lymph flowing, and thereby improves the efficiency of lymphatic pump [136].

It was reported that NO activates PKA and PKG by improving the levels of cAMP and cGMP, respectively, which in turn hyperpolarize the membrane of LSMCs [137–139] and reduce the activity of IP3 [140] on sarcoplasmic reticulum and then reduce

the $[Ca^{2+}]i$. In addition, NO reduces calcium sensitivity of LSMCs via the activation of MLCP; as a result, it reduces the lymphatic contractility, eventually. The regulation of production and release of NO during the process of the lymphatic contraction may be one of the new targets for treating or intervening lymphatic dysfunction diseases.

In consideration of NO regulating lymphatic contraction in a physiological manner, therefore, the expressions of signal pathway molecules of NO-cAMP-PKA and cGMP-PKG were assessed. The results revealed that levels of iNOS, NO, cAMP, cGMP, p-PKA, and p-PKG were gradually increased along with the continued hypotension, and NO donor (L-Arg) and PKA donor (8-Br-cAMP) decreased the contractility and reactivity of shock 0.5-h lymphatics, and these effects were abolished by PKA inhibitor (H-89) and K_{ATP} inhibitor (glibenclamide). Furthermore, NOS antagonist (L-NAME), soluble guanylate cyclase inhibitor (ODQ), and PKG inhibitor (KT-5823) increased the contractility and reactivity of shock 2-h lymphatics, whereas these elevated effects were inhibited by K_{ATP} opener (pinacidil) [19, 141, 142]. In conclusion, these data indicate that NO regulation of lymphatic contractility and reactivity during shock is related to both cAMP-PKA and cGMP-PKG pathways.

5.4.3 Regulatory Effect of RhoA-ROCK-MLCP Pathway on Lymphatic Contractibility Following T/HS

Small G protein Rho and ROCK participate in the regulation of smooth muscle contraction via inhibition of MLCP, regulation of MLC_{20} phosphorylation level, and calcium sensitivity. So, Rho plays an important role in calcium-independent smooth muscle contraction [143]. Hosaka [144] confirmed Rho-ROCK signal pathway participates in the regulation of lymphatic contraction in physiological condition by using ROCK selective inhibitor Y-27632 and MLCP inhibitor OA in rats' ileum lymphatic in vitro. The previous study also found that Rho-ROCK-mediated calcium sensitivity reduction is the main cause that leads to lymphatic myogenic contractility reduction following alcoholism [145].

Our results showed that the contents of RhoA and MLCP exhibit a biphasic change in lymphatic tissue following hemorrhagic shock; RhoA antagonist C3 transferase and the ROCK antagonist Y-27632 significantly reduced the contractility of 0.5 h-shocked lymphatics and reactivity to SP. The RhoA agonist U-46619 increased the contractility and reactivity of 2 h-shocked lymphatics, and Y-27632 and OA restrained the effect of U-46619 [146]. So these results indicate that RhoA-ROCK-MLCP signal pathway participates in the biphasic regulation of lymphatic contractility and reactivity following hemorrhagic shock.

These above results preliminarily revealed the mechanism of lymphatic hypocontractility and hyporeactivity following severe T/HS, thereby providing elementary experimental evidence for prevention and treatment of severe T/HS through regulating the lymphatic function with these intervention targets. Therefore, further, understanding the regulatory mechanism of lymphatic contractile function at different stages of T/HS would have positive significance for the prevention and treatment of severe T/HS.

5.5 Summary and Future Perspectives

Progressions have certainly been achieved in the studies focusing on lymphatic mechanism of severe shock. The lymphatic serves as a double-edged sword in the pathogenesis of hemorrhagic shock. In the early stage of shock and hypotension, the improvement or maintenance of lymphatic function contributes to the effective fluid circulation. Along with continuing hypotension, lymphatic contractility and reactivity are reversely reduced that impairs absorption and transport of lymphatic vessels. These reduced compensatory effects may lead to the refractory shock. Ischemic injury and reperfusion derived from inappropriate fluid resuscitation also destroy the intestinal barrier and lymphatic endothelial barrier. These impaired barriers would result in intestinal lymph reflux containing systemic toxic substances that causes intestinal infection. In this period, the increased lymphatic vessel function will make the infection more severe when the intestinal injury of severe shock is not effectively improved. Collectively, intestinal infection-induced enteric bacteria or endotoxin translocation through intestinal lymphatic pathway is more harmful than intestinal endotoxemia. This would induce multiple organ damages and the occurrence of refractory shock. Therefore, regulation of lymph fluid formation and transport by lymphatic contraction may be useful means to treat refractory shock. MLCK, NO, RhoA, and other signaling molecules are also involved in the regulation of lymphatic constriction and reactivity in severe shock in vitro, but these effects need to be confirmed in animal models.

In the future, we need to seek therapeutic measures for clinical application. Those measures should have capability to regulate the absorption and transport of lymphatic vessels at different stages of shock to improve intestinal injury, reduce hypoxia injury of lymphatic vessels, and restore the function of the lymphatic endothelial barrier. It is required that those measures not only improve the compensation of the lymphatic but also avoid persistent hypotension or intestinal infection during reperfusion by the lymphatic pathway when traumatic shock is treated. However, alterations of lymphatic function during shock and their underlying mechanisms, uncertain regulatory factors, as well as pathophysiological significance of the lymphatic system in many shocks are still unclear and need to be further studied. It is believed that along with more understanding of the lymph formation and transport and its physiological and pathophysiological roles in fluid circulation, lipid metabolism and immune defense, the refractory shock, and lymphatic-related diseases would be treated effectively in the future.

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References

1. Chakraborty S, Gurusamy M, Zawieja DC, Muthuchamy M. Lymphatic filariasis: perspectives on lymphatic remodeling and contractile dysfunction in filarial disease pathogenesis. Microcirculation. 2013;20(5):349–64.
2. Von Der Weid PY, Rehal S. Lymphatic pump function in the inflamed gut. Ann N Y Acad Sci. 2010;1207(Suppl 1):E69–74.
3. Zawieja SD, Wang W, Wu X, Nepiyushchikh ZV, Zawieja DC, Muthuchamy M. Impairments in the intrinsic contractility of mesenteric collecting lymphatics in a rat model of metabolic syndrome. Am J Physiol Heart Circ Physiol. 2012;302(3):H643–53.
4. Jacob M, Kumar P. The challenge in management of hemorrhagic shock in trauma. Med J Armed Forces India. 2014;70(2):163–9.
5. McGhan LJ, Jaroszewski DE. The role of toll-like receptor-4 in the development of multi-organ failure following traumatic haemorrhagic shock and resuscitation. Injury. 2012;43(2):129–36.
6. Duan C, Yang G, Li T, Liu L. Advances in vascular hyporeactivity after shock: the mechanisms and managements. Shock. 2015;44(6):524–34.
7. Deng X, Cao Y, Huby MP, Duan C, Baer L, Peng Z, et al. Adiponectin in fresh frozen plasma contributes to restoration of vascular barrier function after hemorrhagic shock. Shock. 2016;45(1):50–4.
8. Soliman M. Protective effects of estradiol on myocardial contractile function following hemorrhagic shock and resuscitation in rats. Chin Med J. 2015;128(17):2360–4.
9. D'Alessandro A, Moore HB, Moore EE, Wither M, Nemkov T, Gonzalez E, et al. Early hemorrhage triggers metabolic responses that build up during prolonged shock. Am J Physiol Regul Integr Comp Physiol. 2015;308(12):R1034–44.
10. Dekker SE, Sillesen M, Bambakidis T, Jin G, Liu B, Boer C, et al. Normal saline influences coagulation and endothelial function after traumatic brain injury and hemorrhagic shock in pigs. Surgery. 2014;156(3):556–63.
11. Ding N, Chen G, Hoffman R, Loughran PA, Sodhi CP, Hackam DJ, et al. Toll-like receptor 4 regulates platelet function and contributes to coagulation abnormality and organ injury in hemorrhagic shock and resuscitation. Circ Cardiovasc Genet. 2014;7(5):615–24.
12. Zhang Y, Zhang J, Korff S, Ayoob F, Vodovotz Y, Billiar TR. Delayed neutralization of interleukin 6 reduces organ injury, selectively suppresses inflammatory mediator, and partially normalizes immune dysfunction following trauma and hemorrhagic shock. Shock. 2014;42(3):218–27.
13. Pati S, Pilia M, Grimsley JM, Karanikas AT, Oyeniyi B, Holcomb JB, et al. Cellular therapies in trauma and critical care medicine: forging new frontiers. Shock. 2015;44(6):505–23.
14. Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. Am J Phys. 1991;260(2 Pt 2):H305–18.
15. Hubbard W, Keith J, Berman J, Miller M, Scott C, Peck C, et al. 17alpha-Ethynylestradiol-3-sulfate treatment of severe blood loss in rats. J Surg Res. 2015;193(1):355–60.
16. Zhang J. Changes in lymphatic microcirculation during hemorrhagic shock. Zhonghua Yi Xue Za Zhi. 1991;71(1):24–8.
17. Johnston MG, Elias RM, Hayashi A, Nelson W. Role of the lymphatic circulatory system in shock. J Burn Care Rehabil. 1987;8(6):469–74.
18. Higaki A, Kawahara M, Yuge O, Fujii K, Morio M. Mesenteric lymphatic vasomotion following hemorrhage and retransfusion in the rat. Lymphology. 1990;23(4):209–14.
19. Qin LP, Niu CY, Zhao ZG, Zhang J, Zhang YP. Nitric oxide modulates biphasic changes of isolated lymphatic contraction in hemorrhagic shock rats. Sheng Li Xue Bao. 2011;63(4):367–76.
20. Wang HH, Zhang LM, Zhao ZG, Niu CY. Reduction of contractility and reactivity in isolated lymphatics from hemorrhagic shock rats with resuscitation. Acta Cir Bras. 2015;30(3):216–21.

21. Niu CY, Zhao ZG, Zhang YP, Liu ZQ, Zhang J. Lymphatic hyporeactivity and calcium desensitization following hemorrhagic shock. Shock. 2012;37(4):415–23.
22. Zhang LM, Niu CY, Zhao ZG, Qin LP, Si YH, Zhang J. Reactivity to substance P of isolated lymphatics in hemorrhagic shock rat. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2012;28(1):57–61.
23. Ruan X, Shi H, Xia G, Xiao Y, Dong J, Ming F, et al. Encapsulated Bifidobacteria reduced bacterial translocation in rats following hemorrhagic shock and resuscitation. Nutrition. 2007;23(10):754–61.
24. Moore FA, Moore EE, Poggetti RS, Read RA. Postinjury shock and early bacteremia. A lethal combination. Arch Surg. 1992;127(8):893–7. discussion 897–8.
25. Baker JW, Deitch EA, Li M, Berg RD, Specian RD. Hemorrhagic shock induces bacterial translocation from the gut. J Trauma. 1988;28(7):896–906.
26. Deitch EA, Xu D, Kaise VL. Role of the gut in the development of injury- and shock induced SIRS and MODS: the gut-lymph hypothesis, a review. Front Biosci. 2006;11:520–8.
27. Deitch EA. Gut-origin sepsis: evolution of a concept. Surgeon. 2012;10(6):350–6.
28. Leak LV, Cadet JL, Griffin CP, Richardson K. Nitric oxide production by lymphatic endothelial cells in vitro. Biochem Biophys Res Commun. 1995;217(1):96–105.
29. Liu ZY, Casley-Smith JR. The fine structure of the amphibian lymph sac. Lymphology. 1989;22(1):31–5.
30. Moore EE. Mesenteric lymph: the critical bridge between dysfunctional gut and multiple organ failure. Shock. 1998;10(6):415–6.
31. Magnotti LJ, Xu DZ, Lu Q, Deitch EA. Gut-derived mesenteric lymph: a link between burn and lung injury. Arch Surg. 1999;134(12):1333–40. discussion 1340–1.
32. Fanous MY, Phillips AJ, Windsor JA. Mesenteric lymph: the bridge to future management of critical illness. JOP. 2007;8(4):374–99.
33. Deitch EA. Gut lymph and lymphatics: a source of factors leading to organ injury and dysfunction. Ann N Y Acad Sci. 2010;1207(Suppl 1):E103–11.
34. Deitch EA. Role of the gut lymphatic system in multiple organ failure. Curr Opin Crit Care. 2001;7(2):92–8.
35. Magnotti LJ, Upperman JS, Xu DZ, Lu Q, Deitch EA. Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. Ann Surg. 1998;228(4):518–27.
36. Deitch EA, Adams C, Lu Q, Xu DZ. A time course study of the protective effect of mesenteric lymph duct ligation on hemorrhagic shock-induced pulmonary injury and the toxic effects of lymph from shocked rats on endothelial cell monolayer permeability. Surgery. 2001;129(1):39–47.
37. Sambol JT, Xu DZ, Adams CA, Magnotti LJ, Deitch EA. Mesenteric lymph duct ligation provides long term protection against hemorrhagic shock-induced lung injury. Shock. 2000;14(3):416–9. discussion 419–20.
38. Gonzalez RJ, Moore EE, Ciesla DJ, Biffl WL, Johnson JL, Silliman CC. Mesenteric lymph is responsible for post-hemorrhagic shock systemic neutrophil priming. J Trauma. 2001;51(6):1069–72.
39. Xu DZ, Lu Q, Adams CA, Issekutz AC, Deitch EA. Trauma-hemorrhagic shock-induced up-regulation of endothelial cell adhesion molecules is blunted by mesenteric lymph duct ligation. Crit Care Med. 2004;32(3):760–5.
40. Zallen G, Moore EE, Johnson JL, Tamura DY, Ciesla DJ, Silliman CC. Posthemorrhagic shock mesenteric lymph primes circulating neutrophils and provokes lung injury. J Surg Res. 1999;83(2):83–8.
41. Gonzalez RJ, Moore EE, Ciesla DJ, Nieto JR, Johnson JL, Silliman CC. Post-hemorrhagic shock mesenteric lymph activates human pulmonary microvascular endothelium for in vitro neutrophil-mediated injury: the role of intercellular adhesion molecule-1. J Trauma. 2003;54(2):219–23.

42. Deitch EA, Adams CA, Lu Q, Xu DZ. Mesenteric lymph from rats subjected to trauma-hemorrhagic shock are injurious to rat pulmonary microvascular endothelial cells as well as human umbilical vein endothelial cells. Shock. 2001;16(4):290–3.
43. Davidson MT, Deitch EA, Lu Q, Hasko G, Abungu B, Nemeth ZH, et al. Trauma-hemorrhagic shock mesenteric lymph induces endothelial apoptosis that involves both caspase-dependent and caspase-independent mechanisms. Ann Surg. 2004;240(1):123–31.
44. Niu CY, Zhao ZG, Li JC, Chen RH, Zhang J, Zhang YP, et al. Damage effects of shock lymph on the pulmonary micro-vascular endothelial cells of rats. Fen Zi Xi Bao Sheng Wu Xue Bao. 2007;40(2):145–52.
45. Barlos D, Deitch EA, Watkins AC, Caputo FJ, Lu Q, Abungu B, et al. Trauma-hemorrhagic shock-induced pulmonary epithelial and endothelial cell injury utilizes different programmed cell death signaling pathways. Am J Physiol Lung Cell Mol Physiol. 2009;296(3):L404–17.
46. Senthil M, Watkins A, Barlos D, Xu DZ, Lu Q, Abungu B, et al. Intravenous injection of trauma-hemorrhagic shock mesenteric lymph causes lung injury that is dependent upon activation of the inducible nitric oxide synthase pathway. Ann Surg. 2007;246(5):822–30.
47. Wohlauer MV, Moore EE, Harr J, Eun J, Fragoso M, Banerjee A, et al. Cross-transfusion of postshock mesenteric lymph provokes acute lung injury. J Surg Res. 2011;170(2):314–8.
48. Tsan MF, Gao B. Endogenous ligands of toll-like receptors. J Leukoc Biol. 2004;76(3):514–9.
49. Reino DC, Pisarenko V, Palange D, Doucet D, Bonitz RP, Lu Q, et al. Trauma hemorrhagic shock-induced lung injury involves a gut-lymph-induced TLR4 pathway in mice. PLoS One. 2011;6(8):e14829.
50. Chatpun S, Cabrales P. Cardiac systolic function recovery after hemorrhage determines survivability during shock. J Trauma. 2011;70(4):787–93.
51. Piquereau J, Godin R, Deschenes S, Bessi VL, Mofarrahi M, Hussain SN, et al. Protective role of PARK2/Parkin in sepsis-induced cardiac contractile and mitochondrial dysfunction. Autophagy. 2013;9(11):1837–51.
52. Zaky A, Deem S, Bendjelid K, Treggiari MM. Characterization of cardiac dysfunction in sepsis: an ongoing challenge. Shock. 2014;41(1):12–24.
53. Zhao ZG, Niu CY, Chen RH, Zhang YP, Zhang J, Liu YK, et al. Effect of intestinal lymphatic pathway on free radical and inflammatory mediator of myocardium in shock rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2007;23(4):385–9.
54. Sambol JT, Lee MA, Caputo FJ, Kawai K, Badami C, Kawai T, et al. Mesenteric lymph duct ligation prevents trauma/hemorrhage shock-induced cardiac contractile dysfunction. J Appl Physiol (1985). 2009;106(1):57–65.
55. Du HB, Wang SH, Zhao ZG, Niu CY. Post-hemorrhagic shock mesenteric lymph is an important contributor to cardiac dysfunction following hemorrhagic shock. Acta Cir Bras. 2015;30(6):439–44.
56. Sambol JT, Lee MA, Jiang M, Dosi G, Dong W, Deitch EA, et al. Mesenteric lymph from rats with trauma-hemorrhagic shock causes abnormal cardiac myocyte function and induces myocardial contractile dysfunction. J Appl Physiol (1985). 2011;111(3):799–807.
57. Wohlauer MV, Sauaia A, Moore EE, Burlew CC, Banerjee A, Johnson J. Acute kidney injury and posttrauma multiple organ failure: the canary in the coal mine. J Trauma Acute Care Surg. 2012;72(2):373–8. discussion 379–80.
58. Rohrig R, Ronn T, Lendemans S, Feldkamp T, de Groot H, Petrat F. Adverse effects of resuscitation with lactated ringer compared with ringer solution after severe hemorrhagic shock in rats. Shock. 2012;38(2):137–45.
59. Yang HY, Yen TH, Lin CY, Chen YC, Pan MJ, Lee CH, et al. Early identification of leptospirosis as an ignored cause of multiple organ dysfunction syndrome. Shock. 2012;38(1):24–9.
60. Niu CY, Zhao ZG, Ye YL, Hou YL, Zhang YP. Mesenteric lymph duct ligation against renal injury in rats after hemorrhagic shock. Ren Fail. 2010;32(5):584–91.
61. Stringham JR, Moore EE, Gamboni F, Harr JN, Fragoso M, Chin TL, et al. Mesenteric lymph diversion abrogates 5-lipoxygenase activation in the kidney following trauma and hemorrhagic shock. J Trauma Acute Care Surg. 2014;76(5):1214–21.

62. Zhao ZG, Zhu HX, Zhang LM, Zhang YP, Niu CY. Mesenteric lymph drainage alleviates acute kidney injury induced by hemorrhagic shock without resuscitation. ScientificWorldJournal. 2014;2014:720836.
63. Han B, Zhao ZG, Zhang LM, Li SG, Niu CY. Hydrogen sulfide in posthemorrhagic shock mesenteric lymph drainage alleviates kidney injury in rats. Braz J Med Biol Res. 2015;48(7):622–8.
64. Zhao ZG, Niu CY, Qiu JF, Chen XD, Li JC. Effect of mesenteric lymph duct ligation on gene expression profiles of renal tissue in hemorrhagic shock rats with fluid resuscitation. Ren Fail. 2014;36(2):271–7.
65. Zhao ZG, Zhang LM, Lv YZ, Si YH, Niu CY, Li JC. Changes in renal tissue proteome induced by mesenteric lymph drainage in rats after hemorrhagic shock with resuscitation. Shock. 2014;42(4):350–5.
66. Douzinas EE. Hemorrhagic shock resuscitation: a critical issue on the development of post-traumatic multiple organ failure. Crit Care Med. 2012;40(4):1348–9.
67. Zhou R, Ding XL, Liu LM. Ryanodine receptor 2 contributes to hemorrhagic shock-induced bi-phasic vascular reactivity in rats. Acta Pharmacol Sin. 2014;35(11):1375–84.
68. Lei Y, Peng X, Liu L, Dong Z, Li T. Beneficial effect of cyclosporine A on traumatic hemorrhagic shock. J Surg Res. 2015;195(2):529–40.
69. Liu S, Li T, Yang G, Hu Y, Xiao X, Xu J, et al. Protein markers related to vascular responsiveness after hemorrhagic shock in rats. J Surg Res. 2015;196(1):149–58.
70. Zhao KS, Liu J, Yang GY, Jin C, Huang Q, Huang X. Peroxynitrite leads to arteriolar smooth muscle cell membrane hyperpolarization and low vasoreactivity in severe shock. Clin Hemorheol Microcirc. 2000;23(2–4):259–67.
71. Zhao KS, Huang X, Liu J, Huang Q, Jin C, Jiang Y, et al. New approach to treatment of shock--restitution of vasoreactivity. Shock. 2002;18(2):189–92.
72. Pan BX, Zhao GL, Huang XL, Jin JQ, Zhao KS. Peroxynitrite induces arteriolar smooth muscle cells membrane hyperpolarization with arteriolar hyporeactivity in rats. Life Sci. 2004;74(10):1199–210.
73. Pan BX, Zhao GL, Huang XL, Zhao KS. Mobilization of intracellular calcium by peroxynitrite in arteriolar smooth muscle cells from rats. Redox Rep. 2004;9(1):49–55.
74. Zhao KS. Hemorheologic events in severe shock. Biorheology. 2005;42(6):463–77.
75. Zhao ZG, Niu CY, Wei YL, Zhang YP, Si YH, Zhang J. Mesenteric lymph return is an important contributor to vascular hyporeactivity and calcium desensitization after hemorrhagic shock. Shock. 2012;38(2):186–95.
76. Li T, Fang Y, Yang G, Zhu Y, Xu J, Liu L. The mechanism by which RhoA regulates vascular reactivity after hemorrhagic shock in rats. Am J Physiol Heart Circ Physiol. 2010;299(2):H292–9.
77. Li T, Yang G, Xu J, Zhu Y, Liu L. Regulatory effect of Rac1 on vascular reactivity after hemorrhagic shock in rats. J Cardiovasc Pharmacol. 2011;57(6):656–65.
78. Li T, Fang Y, Yang G, Xu J, Zhu Y, Liu L. Effects of the balance in activity of RhoA and Rac1 on the shock-induced biphasic change of vascular reactivity in rats. Ann Surg. 2011;253(1):185–93.
79. Liu L, Zang J, Chen X, Yang G, Zhu Y, Wu Y, et al. Role of miR-124 and miR-141 in the regulation of vascular reactivity and the relationship to RhoA and Rac1 after hemorrhage and hypoxia. Am J Physiol Heart Circ Physiol. 2016;310(2):H206–16.
80. Li T, Liu L, Xu J, Yang G, Ming J. Changes of Rho kinase activity after hemorrhagic shock and its role in shock-induced biphasic response of vascular reactivity and calcium sensitivity. Shock. 2006;26(5):504–9.
81. Li T, Liu L, Liu J, Ming J, Xu J, Yang G, et al. Mechanisms of Rho kinase regulation of vascular reactivity following hemorrhagic shock in rats. Shock. 2008;29(1):65–70.
82. Yang G, Liu L, Xu J, Li T. Effect of arginine vasopressin on vascular reactivity and calcium sensitivity after hemorrhagic shock in rats and its relationship to Rho-kinase. J Trauma. 2006;61(6):1336–42.

83. Xu J, Yang GM, Li T, Ming J, Chen W, Zhang Y, et al. The regulatory effect of protein kinase C epsilon on vascular reactivity and calcium sensitivity during hemorrhagic shock in rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2008;20(3):144–7.
84. Fang Y, Li T, Fan X, Zhu Y, Liu L. Beneficial effects of activation of PKC on hemorrhagic shock in rats. J Trauma. 2010;68(4):865–73.
85. Yang G, Li T, Xu J, Liu L. PKC plays an important mediated effect in arginine vasopressin induced restoration of vascular responsiveness and calcium sensitization following hemorrhagic shock in rats. Eur J Pharmacol. 2010;628(1–3):148–54.
86. Yang GM, Li T, Xu J, Ming J, Liu LM. Effect of arginine vasopressin on vascular reactivity and calcium sensitivity of vascular smooth muscle and its relationship to protein kinase C following hemorrhagic shock in rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2008;20(3):139–43.
87. Xu J, Li T, Yang G, Liu L. Pinacidil pretreatment improves vascular reactivity after shock through PKCalpha and PKCepsilon in rats. J Cardiovasc Pharmacol. 2012;59(6):514–22.
88. Xu J, Lan D, Yang G, Li T, Liu L. Hemorrhagic preconditioning improves vascular reactivity after hemorrhagic shock by activation of PKCalpha and PKCepsilon via the adenosine A1 receptor in rats. J Trauma Acute Care Surg. 2013;74(5):1266–74.
89. Li T, Liu LM, Liu JC. Regulatory effect of protein kinase C and protein kinase G on calcium sensitivity of vascular smooth muscle cells following hemorrhagic shock. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2007;19(5):257–60.
90. Yang G, Li T, Xu J, Peng X, Liu L. Mitogen-activated protein kinases regulate vascular reactivity after hemorrhagic shock through myosin light chain phosphorylation pathway. J Trauma Acute Care Surg. 2013;74(4):1033–43.
91. Yang G, Xu J, Li T, Ming J, Chen W, Liu L. Role of V1a receptor in AVP-induced restoration of vascular hyporeactivity and its relationship to MLCP-MLC20 phosphorylation pathway. J Surg Res. 2010;161(2):312–20.
92. Zhao Z, Si Y, Zhang Y, Du S, Zhang L, Niu C. Postshock mesenteric lymph drainage ameliorates vascular reactivity and calcium sensitivity through RhoA. J Surg Res. 2014;186(1):304–9.
93. Zhang YP, Niu CY, Zhao ZG, Zhang LM, Si YH. Myosin light chain kinase is necessary for post-shock mesenteric lymph drainage enhancement of vascular reactivity and calcium sensitivity in hemorrhagic-shocked rats. Braz J Med Biol Res. 2013;46(7):574–9.
94. Niu CY, Zhao ZG, Wei YL, Zhang YP, Zhang J. Involvement of protein kinase C in enhancement of vascular calcium sensitivity by blocking mesenteric lymph return in hemorrhagic shock rats. Sheng Li Xue Bao. 2012;64(2):213–9.
95. Zhao ZG, Wei YL, Niu CY, Zhang YP, Zhang LM, Jiang LN. Role of protein kinase G on the post-shock mesenteric lymph blockage ameliorating vascular calcium sensitivity. Acta Cir Bras. 2013;28(7):537–42.
96. Zhao ZG, Niu CY, Wei YL, Zhang YP, Si YH, Zhang J. Role of rho kinase in blocking the return of mesenteric lymph to improve vascular calcium sensitivity in hemorrhagic shock rats. Chin J Pathophysiol. 2012;1:11–5.
97. Song R, Bian H, Wang X, Huang X, Zhao KS. Mitochondrial injury underlies hyporeactivity of arterial smooth muscle in severe shock. Am J Hypertens. 2011;24(1):45–51.
98. Wang X, Song R, Chen Y, Zhao M, Zhao KS. Polydatin--a new mitochondria protector for acute severe hemorrhagic shock treatment. Expert Opin Investig Drugs. 2013;22(2):169–79.
99. Sun GX, Guo YX, Du HB, Zhang LM, Zhao ZG, Liu SJ, et al. Role of post-hemorrhagic shock mesenteric lymph in enhancement of vascular permeability. Chin J Pathophysiol. 2014;30(8):1506–36.
100. Lu Q, Xu DZ, Davidson MT, Hasko G, Deitch EA. Hemorrhagic shock induces endothelial cell apoptosis, which is mediated by factors contained in mesenteric lymph. Crit Care Med. 2004;32(12):2464–70.
101. Komarova Y, Malik AB. Regulation of endothelial permeability via paracellular and transcellular transport pathways. Annu Rev Physiol. 2010;72:463–93.
102. Dejana E, Tournier-Lasserve E, Weinstein BM. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. Dev Cell. 2009;16(2):209–21.

103. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006;86(1):279–367.
104. Komarova YA, Kruse K, Mehta D, Malik AB. Protein interactions at endothelial junctions and signaling mechanisms regulating endothelial permeability. Circ Res. 2017;120(1):179–206.
105. Wallez Y, Huber P. Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. Biochim Biophys Acta. 2008;1778(3):794–809.
106. Sun GX, Guo YX, Zhang YP, Zhang LM, Zhao ZG, Niu CY. Posthemorrhagic shock mesenteric lymph enhances monolayer permeability via F-actin and VE-cadherin. J Surg Res. 2016;203(1):47–55.
107. Angele MK, Chaudry IH. Surgical trauma and immunosuppression: pathophysiology and potential immunomodulatory approaches. Langenbeck's Arch Surg. 2005;390(4):333–41.
108. Hostmann A, Jasse K, Schulze-Tanzil G, Robinson Y, Oberholzer A, Ertel W, et al. Biphasic onset of splenic apoptosis following hemorrhagic shock: critical implications for Bax, Bcl-2, and Mcl-1 proteins. Crit Care. 2008;12(1):R8.
109. Barkhausen T, Frerker C, Putz C, Pape HC, Krettek C, van Griensven M. Depletion of NK cells in a murine polytrauma model is associated with improved outcome and a modulation of the inflammatory response. Shock. 2008;30(4):401–10.
110. Kawasaki T, Suzuki T, Choudhry MA, Bland KI, Chaudry IH. Salutary effects of 17beta-estradiol on Peyer's patch T cell functions following trauma-hemorrhage. Cytokine. 2010;51(2):166–72.
111. Grootjans J, Hodin CM, de Haan JJ, Derikx JP, Rouschop KM, Verheyen FK, et al. Level of activation of the unfolded protein response correlates with Paneth cell apoptosis in human small intestine exposed to ischemia/reperfusion. Gastroenterology. 2011;140(2):529–539.e3.
112. Kim JY, Hong YS, Choi SH, Yoon YH, Moon SW, Lee SW. Effect of hypertonic saline on apoptosis of polymorphonuclear cells. J Surg Res. 2012;178(1):401–8.
113. Tiesi G, Reino D, Mason L, Palange D, Tomaio JN, Deitch EA. Early trauma-hemorrhage-induced splenic and thymic apoptosis is gut-mediated and toll-like receptor 4-dependent. Shock. 2013;39(6):507–13.
114. Liu H, Zhao ZG, Xing LQ, Zhang LM, Niu CY. Post-shock mesenteric lymph drainage ameliorates cellular immune function in rats following hemorrhagic shock. Inflammation. 2015;38(2):584–94.
115. Liu H, Xing LQ, Zhao ZG, Niu CY. Mesenteric lymph drainage alleviates spleen injury in hemorrhagic shock rats. Chin J Pathophysiol. 2013;29(8):1496–501.
116. Adams CA Jr, Xu DZ, Lu Q, Deitch EA. Factors larger than 100 kd in post-hemorrhagic shock mesenteric lymph are toxic for endothelial cells. Surgery. 2001;129(3):351–63.
117. Kaiser VL, Sifri ZC, Dikdan GS, Berezina T, Zaets S, Lu Q, et al. Trauma-hemorrhagic shock mesenteric lymph from rat contains a modified form of albumin that is implicated in endothelial cell toxicity. Shock. 2005;23(5):417–25.
118. Kaiser VL, Sifri ZC, Senthil M, Dikdan GS, Lu Q, Xu DZ, et al. Albumin peptide: a molecular marker for trauma/hemorrhagic-shock in rat mesenteric lymph. Peptides. 2005;26(12):2491–9.
119. Jordan JR, Moore EE, Damle SS, Eckels P, Johnson JL, Roach JP, et al. Gelsolin is depleted in post-shock mesenteric lymph. J Surg Res. 2007;143(1):130–5.
120. Peltz ED, Moore EE, Zurawel AA, Jordan JR, Damle SS, Redzic JS, et al. Proteome and system ontology of hemorrhagic shock: exploring early constitutive changes in postshock mesenteric lymph. Surgery. 2009;146(2):347–57.
121. Fang JF, Shih LY, Yuan KC, Fang KY, Hwang TL, Hsieh SY. Proteomic analysis of post-hemorrhagic shock mesenteric lymph. Shock. 2010;34(3):291–8.
122. Mittal A, Middleditch M, Ruggiero K, Loveday B, Delahunt B, Jullig M, et al. Changes in the mesenteric lymph proteome induced by hemorrhagic shock. Shock. 2010;34(2):140–9.
123. D'Alessandro A, Dzieciatkowska M, Peltz ED, Moore EE, Jordan JR, Silliman CC, et al. Dynamic changes in rat mesenteric lymph proteins following trauma using label-free mass spectrometry. Shock. 2014;42(6):509–17.

124. Dzieciatkowska M, D'Alessandro A, Moore EE, Wohlauer M, Banerjee A, Silliman CC, et al. Lymph is not a plasma ultrafiltrate: a proteomic analysis of injured patients. Shock. 2014;42(6):485–98.
125. Gonzalez RJ, Moore EE, Biffl WL, Ciesla DJ, Silliman CC. The lipid fraction of post-hemorrhagic shock mesenteric lymph (PHSML) inhibits neutrophil apoptosis and enhances cytotoxic potential. Shock. 2000;14(3):404–8.
126. Gonzalez RJ, Moore EE, Ciesla DJ, Biffl WL, Offner PJ, Silliman CC. Phospholipase A(2)--derived neutral lipids from posthemorrhagic shock mesenteric lymph prime the neutrophil oxidative burst. Surgery. 2001;130(2):198–203.
127. Gonzalez RJ, Moore EE, Ciesla DJ, Meng X, Biffl WL, Silliman CC. Post-hemorrhagic shock mesenteric lymph lipids prime neutrophils for enhanced cytotoxicity via phospholipase A2. Shock. 2001;16(3):218–22.
128. Morishita K, Aiboshi J, Kobayashi T, Mikami S, Yokoyama Y, Ogawa K, et al. Lipidomics analysis of mesenteric lymph after trauma and hemorrhagic shock. J Trauma Acute Care Surg. 2012;72(6):1541–7.
129. Qin X, Dong W, Sharpe SM, Sheth SU, Palange DC, Rider T, et al. Role of lipase-generated free fatty acids in converting mesenteric lymph from a noncytotoxic to a cytotoxic fluid. Am J Physiol Gastrointest Liver Physiol. 2012;303(8):G969–78.
130. Davis MJ, Lane MM, Davis AM, Durtschi D, Zawieja DC, Muthuchamy M, et al. Modulation of lymphatic muscle contractility by the neuropeptide substance P. Am J Physiol Heart Circ Physiol. 2008;295(2):H587–97.
131. Nepiyushchikh ZV, Chakraborty S, Wang W, Davis MJ, Zawieja DC, Muthuchamy M. Differential effects of myosin light chain kinase inhibition on contractility, force development and myosin light chain 20 phosphorylation of rat cervical and thoracic duct lymphatics. J Physiol. 2011;589(Pt 22):5415–29.
132. Qin LP, Niu CY, Zhao ZG, Zhang J, Zhang YP. Substance P enhances pump function of isolated lymphatics from hemorrhagic shock rats. Chin J Pathophysiol. 2011;27(7):1323–8.
133. Zhang YP, Niu CY, Zhao ZG, Qin LP, Si YH, Zhang LM, et al. Role of myosin-light-chain kinase in biphasic contractile activity of lymphatics isolated from hemorrhagic shock rats. Chin J Pathophysiol. 2012;28(4):589–94.
134. Kawai Y, Yokoyama Y, Kaidoh M, Ohhashi T. Shear stress-induced ATP-mediated endothelial constitutive nitric oxide synthase expression in human lymphatic endothelial cells. Am J Physiol Cell Physiol. 2009;298(3):C647–55.
135. Bohlen HG, Wang W, Gashev A, Gasheva O, Zawieja D. Phasic contractions of rat mesenteric lymphatics increase basal and phasic nitric oxide generation in vivo. Am J Physiol Heart Circ Physiol. 2009;297(4):H1319–28.
136. Gasheva OY, Zawieja DC, Gashev AA. Contraction-initiated NO-dependent lymphatic relaxation: a self-regulatory mechanism in rat thoracic duct. J Physiol. 2006;575(Pt 3):821–32.
137. von der Weid PY, Zhao J, Van Helden DF. Nitric oxide decreases pacemaker activity in lymphatic vessels of guinea pig mesentery. Am J Physiol Heart Circ Physiol. 2001;280(6):H2707–16.
138. Bridenbaugh EA, Gashev AA, Zawieja DC. Lymphatic muscle: a review of contractile function. Lymphat Res Biol. 2003;1(2):147–58.
139. von der Weid PY. ATP-sensitive K+ channels in smooth muscle cells of guinea-pig mesenteric lymphatics: role in nitric oxide and beta-adrenoceptor agonist-induced hyperpolarizations. Br J Pharmacol. 1998;125(1):17–22.
140. Wang W, Nepiyushchikh Z, Zawieja DC, Chakraborty S, Zawieja SD, Gashev AA, et al. Inhibition of myosin light chain phosphorylation decreases rat mesenteric lymphatic contractile activity. Am J Physiol Heart Circ Physiol. 2009;297(2):H726–34.
141. Zhang LM, Qin LP, Zhang YP, Zhao ZG, Niu CY. Nitric oxide regulates the lymphatic reactivity following hemorrhagic shock through Atp-sensitive potassium Channel. Shock. 2016;45(6):668–76.

142. Zhang LM, Niu CY, Zhao ZG, Si YH, Zhang YP. ATP-sensitive potassium channel involved in modulation of nitride oxide regulating contractile activity of isolated lymphatics from hemorrhagic shock rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2012;24(8):457–60.
143. Liao MH, Shih CC, Tsao CM, Chen SJ, Wu CC. RhoA/Rho-kinase and nitric oxide in vascular reactivity in rats with endotoxaemia. PLoS One. 2013;8(2):e56331.
144. Hosaka K, Mizuno R, Ohhashi T. Rho-Rho kinase pathway is involved in the regulation of myogenic tone and pump activity in isolated lymph vessels. Am J Physiol Heart Circ Physiol. 2003;284(6):H2015–25.
145. Souza-Smith FM, Molina PE, Breslin JW. Reduced RhoA activity mediates acute alcohol intoxication-induced inhibition of lymphatic myogenic constriction despite increased cytosolic [Ca(2+)]. Microcirculation. 2013;20(5):377–84.
146. Si YH, Niu CY, Zhao ZG, Zhang LM, Zhang YP. Role of RhoA in regulating the pump function of isolated lymphatics from hemorrhagic shock rats. Shock. 2013;40(1):49–58.

Cardiac Autonomic Nervous System and Sepsis-Induced Cardiac Dysfunction

6

Huadong Wang

Abstract

Cardiac dysfunction is one of the main predictors of poor prognosis in septic patients. Although it has been investigated for more than 30 years, the mechanisms for sepsis-induced cardiac dysfunction are not completely understood, and no specific, effective treatment exists. Traditionally, sepsis-induced cardiac dysfunction was defined as a reversible decrease in ejection fraction of both ventricles with ventricular dilation and depressed response to fluid resuscitation and catecholamines. Many studies have demonstrated that autonomic nervous system imbalance, characterized by sympathetic overactivation and vagal suppression, contributes to the pathogenesis of sepsis-induced cardiac dysfunction. Thus, this kind of cardiac dysfunction can perhaps be best described as a sepsis-induced cardiac autonomic dysfunction as well as an intrinsic systolic and diastolic dysfunction of the whole heart, which is characterized by tachycardia, strongly decreased heart rate variability, and depressed intrinsic systolic and diastolic function of both ventricles. This review will summarize our current knowledge of sepsis-induced cardiac dysfunction, with a special focus on the role of autonomic dysfunction.

Sepsis, a life-threatening syndrome caused by a dysregulated host response to infection, may lead to septic shock and multiple organ dysfunction and finally to death. It is among the most expensive diseases treated in US hospitals and a leading cause of mortality in the intensive care unit worldwide [1]. Although epidemiologic

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studies demonstrated that mortality related to sepsis dropped over the past several decades because of advances in medical management, the incidence of sepsis has increased [2]. Based on clinical data, sepsis-related mortality or discharge to hospice has remained stable between 2009 and 2014 in the USA [3]. According to Sepsis-3 definition, recent study demonstrated that 47% of septic patients had septic shock and in-hospital mortality reached 38.9% [4]. The cardiovascular system plays a pivotal role in the pathogenesis of septic shock; a large body of evidence has demonstrated cardiac dysfunction is a common complication in septic patients and around 50% of septic patients exhibit signs of cardiac dysfunction, including systolic and diastolic dysfunction and cardiac autonomic dysfunction, which is associated with increased morbidity and mortality of septic patients [5–9]. In a long-term fluid-resuscitated rat model of peritonitis, myocardial dysfunction could be detected at the early stage of sepsis, and this early functional change in the heart predicted outcome [10]. In particular, maintenance of cardiac function by specific activation of cardiomyocyte phosphoinositide-3-kinase (PI3K)/Akt-dependent signaling attenuated the cardiac dysfunction and in turn decreased mortality in cecal ligation and puncture (CLP)-induced sepsis [11]. Therefore, sepsis and septic shock are still major public health problems, cardiac dysfunction plays a prominent role in the pathology of sepsis, and maintenance of cardiac function could be of crucial importance in improving the prognosis in patients with sepsis. Nevertheless, there are no specific therapies for sepsis-induced cardiac dysfunction (SICD) to date.

Here, we will discuss the mechanism of SICD with a special focus on the role of cardiac autonomic nervous system and hope to provide further insights for the treatment of this kind of cardiac dysfunction.

6.1 The Concept of Sepsis-Induced Cardiac Dysfunction

Although a large number of studies have demonstrated evidence of cardiac dysfunction during sepsis over the last 50 years, there is a lack of the universally accepted definition of SICD due to the limitation of methodology in evaluating cardiac intrinsic contractile and diastolic function during sepsis in clinic practice [12, 13]. In 1981, Calvin et al. first described myocardial function in adequately volume-resuscitated patients with sepsis using hemodynamic monitoring techniques and radionuclide angiography. They found that septic patients were shown to have a normal left ventricular ejection fraction (EF), which was markedly higher than that of critically ill cardiac patients. Furthermore, the average left ventricular velocity of ejection, a noninvasive evaluation of left ventricular contractility, increased in septic patients compared to normal subjects. Accordingly, they concluded that myocardial contractile dysfunction was not a major feature at early stage of sepsis in septic patients [14]. Three years later, Packer et al. examined the cardiac function using serial radionuclide cineangiographic and hemodynamic evaluations in patients with septic shock; they demonstrated that the survivors of septic patients had a reduced mean initial left ventricle EF and an increased mean end-systolic and end-diastolic volume despite normal or elevated cardiac index, all of which returned to normal

between 7 and 10 days after the onset of septic shock [15]. Since then, the concept of sepsis-induced cardiac dysfunction emerged. It has been described as a reversible reduction in EF of both ventricles with ventricular dilation and depressed response to fluid resuscitation and catecholamines in numerous clinical studies [16, 17]. However, Packer et al. also found that nonsurvivors of septic shock had normal initial EF and ventricular volumes did not alter during serial studies [15]. A recent meta-analysis showed that there was no association between left ventricle and right ventricle systolic dysfunction, identified by decreased EF, and mortality in patients with severe sepsis or septic shock; there were no significant differences in left ventricular EF, right ventricular EF, and right ventricular dimensions between the survivors and nonsurvivors of septic patients [18]. It is now well-known that left ventricular EF, widely used to evaluate left ventricular contractility in the clinical setting, is a load-dependent clinical parameter; it reflects the interaction between the left ventricle and the left ventricular afterload. When arterial tone and left ventricular afterload are severely decreased, left ventricular EF may be normal despite the existence of seriously impaired left ventricular intrinsic contractility. Thus, EF is not a reliable indicator of the intrinsic myocardial function in intact hearts of septic patients with largely different loading conditions [7, 12]. With the advances in tissue Doppler imaging and hemodynamic monitoring techniques, the intrinsic myocardial systolic and diastolic dysfunction of the whole heart were observed in septic patients. Recently, left ventricular peak systolic strain and peak systolic strain rate, quantified by echocardiographic speckle-tracking imaging, have been proposed as novel indicators of intrinsic myocardial systolic function. It was demonstrated that left ventricular peak systolic strain is less dependent than EF to loading conditions [19] and might be useful in the early detection of myocardial dysfunction in sepsis [20]. Compared to non-septic shock patients with normal EF, left ventricular global and right ventricular free wall strain were decreased in septic shock patients with normal EF; 50% of septic patients with preserved left ventricular EF had a depressed left ventricular global strain [20]. On the other hand, left ventricular diastolic function can be determined by tissue Doppler assessment of mitral annular early-diastolic peak velocity (e′ wave), a load-independent parameter of diastolic dysfunction, and the ratio of pulsed-wave Doppler early mitral inflow velocity (E) to e′ wave (E/e′) [21]. More recent studies demonstrated that about 50% of septic patients were found to have left ventricular diastolic dysfunction according to low e′ wave and high E/e′ ratio, which are reliable predictors of diastolic dysfunction. Furthermore, left ventricular diastolic dysfunction, as shown by decreased e′ wave and higher E/e′ ratio, is strongly associated with mortality in septic patients [8, 22]. Evidently, left ventricular diastolic dysfunction is very common in septic patients, and it contributes to increased mortality. According to these observations, it is, more recently, proposed that sepsis-induced cardiac dysfunction can be defined as an intrinsic, global (systolic and diastolic) dysfunction of both the left and right sides of the heart [12]. However, a real problem in sepsis-induced cardiac dysfunction is not only myocardial dysfunction but also the impaired modulation of cardiac function due to extensive changes in the cardiovascular autonomic nervous system. Many studies have shown that cardiac autonomic dysfunction exists in septic

patients, as manifested by an increased heart rate and a strong reduction in sympathetically and vagally mediated heart rate variability (HRV). The loss of HRV in sepsis plays a key role in the sepsis-induced cardiac dysfunction, and depressed heart rate variability indicated a high probability of progression to multiple organ failure with poor prognosis [23–25]. In addition, in neonatal septic patients, HRV analysis is more sensitive than cardiac output and mean arterial pressure in the earlier identification of sepsis [26]. For these reasons, sepsis-induced cardiac dysfunction can perhaps be best defined as a sepsis-evoked cardiac autonomic dysfunction as well as an intrinsic systolic and diastolic dysfunction of the whole heart, which is characterized by tachycardia, strongly decreased HRV, and depressed intrinsic systolic and diastolic function of both ventricles.

6.2 Cardiac Autonomic Innervation

As shown in Fig. 6.1, cerebral networks passing through the hypothalamus and brainstem control sympathetic and parasympathetic output to the cardiovascular system and adrenal gland. The heart is extensively innervated by the sympathetic and parasympathetic nerves. Sympathetic postganglionic nerves travel from the base of the heart into the myocardium along the epicardial vascular structures. These sympathetic postganglionic nerves originate in sympathetic neurons in the cervical and thoracic sympathetic ganglions, in which sympathetic preganglionic nerves communicate with spinal cord. Parasympathetic nervous innervation to the

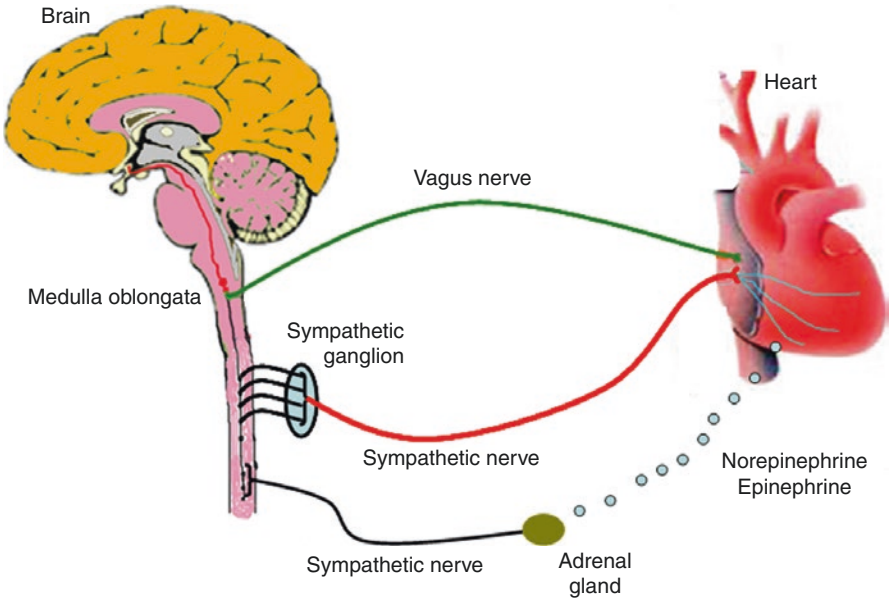

Fig. 6.1 Cardiac autonomic innervation

heart comes predominantly from the parasympathetic neurons in the cardiac ganglia located on the epicardium, whose preganglionic parasympathetic nerves come from the vagus nerves. Once arriving into the pericardial sac, sympathetic postganglionic and preganglionic parasympathetic nerves together with parasympathetic ganglia and parasympathetic postganglionic nerves form a complex autonomic network, cardiac plexus, to innervate the atria, the ventricles, and the special conduction system and then control cardiac performance, such as heart rate, ventricular function, and natriuretic peptide secretion. In addition, the atria (particularly in the sinoatrial node) and ventricles also contain intrinsic cardiac neurons. In the heart, sympathetic nerves release norepinephrine, which causes an increase in heart rate, myocardial conductivity, and contractility to increase cardiac output, myocardial work, and myocardial oxygen consumption, whereas parasympathetic nerves secrete neurotransmitter acetylcholine, which results in a decrease in heart rate to reduce myocardial oxygen consumption and increase coronary diastolic perfusion times. Moreover, preganglionic sympathetic nerves also innervate the adrenal gland; its activation stimulates the adrenal medulla to secrete catecholamines (adrenaline and norepinephrine) into the circulation. This sympathoadrenal activation strongly enhances myocardial contractility and, at the same time, results in the skeletal muscle vasodilation and generalized peripheral vasoconstriction [27, 28].

6.3 Cardiac Autonomic Dysfunction and the Pathogenesis of SICD

During sepsis, various pathogen-associated molecular patterns (PAMPs) from infecting organisms, such as lipopolysaccharide (LPS) released by Gram-negative bacteria, and endogenous damage-associated molecular patterns (DAMPs), including extracellular histones and high-mobility group box 1 (HMGB1), stimulate toll-like receptors (TLRs) on immune cells, cardiomyocytes, cardiac fibroblasts, and endothelial cells. This induces the production of nitric oxide and multiple proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1 and IL-6, through activating transcript factor nuclear factor (NF)-κB, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases 1/2 (ERK1/ERK2), p38 mitogen-activated protein kinase (MAPK), interferon regulatory factor 3, and signal transducer and activator of transcription (STAT) signaling. Many studies have demonstrated that proinflammatory cytokines, nitric oxide, and other inflammatory mediators play a significant role in the pathogenesis of sepsis-induced cardiac dysfunction via provoking cardiomyocyte calcium regulation disorder, the decrease in myocardial fatty acid oxidation, mitochondrial dysfunction, oxidative stress, apoptosis, endothelial dysfunction, and autonomic nervous system dysregulation [29–31]. Evidently, sepsis-induced cardiac dysfunction is a result of the interaction of many factors, including inflammation, metabolism, and cardiac autonomic regulation.

6.3.1 Cardiac Autonomic Nervous System Dysregulation

At present, scientific research into cardiac dysfunction during sepsis focuses on myocardial depression. Less attention has been paid to the strongly impaired regulation of cardiac function due to the cardiovascular autonomic nervous system dysfunction. Increasing evidence indicates that autonomic nervous dysfunction contributes substantially to the development of sepsis-induced cardiac dysfunction. In sepsis, cardiac autonomic dysregulation is characterized by increased heart rate, high levels of circulatory catecholamines, decreased cardiac responsiveness to intrinsic catecholamines, decreased density of myocardial adrenoceptors, and disrupted signal transduction (e.g., reduced levels of stimulatory G-protein and increased expression of inhibitory G-protein) in cardiomyocytes as well as reduced HRV [30, 31]. HRV represents the complex interaction between cardiac pacemaker cells and autonomic nervous system. The loss of sympathetically and vagally mediated HRV is a risk factor for death in septic patients [24]. It has been demonstrated that the depressed HRV in sepsis is most likely due to a mitigated heart rate modulation either by the rate-increasing sympathetic activity, the rate-decreasing vagal activity, or both. In a postmortem study, greater degrees of ischemia and neuronal and glial apoptosis were observed in cardiovascular autonomic centers from patients with septic shock than those without septic shock [32]. In septic rats, increased levels of inflammation and oxidative injury were found in the cardiovascular autonomic centers (brainstem and hypothalamus), but not in the prefrontal cortex, which is not directly involved in control of the autonomic nervous system. Increased sympathetic output and increased left ventricular contractility were observed in septic rats with good prognosis. There was a significant negative correlation between parasympathetic outflow and contractility [25]. These results suggest that increased inflammation in brain centers for cardiac autonomic control may be associated with sympathovagal imbalance and depressed cardiac contractility. In 2009, Ramchandra et al. also demonstrated that administration of *Escherichia coli* induced septic shock in conscious sheep, evidenced by hypotension as well as an increase in heart rate associated with similar changes in cardiac sympathetic nerve activity. The high correlation between the changes in cardiac sympathetic nerve activity and HR indicates that increased sympathetic drive to the heart may contribute to tachycardia during septic shock [33]. It is well-known that sepsis induces hypotension and the normal compensatory response to hypotension is an immediate baroreceptor sensing, which increases sympathetic outflow to both the heart and peripheral vessels and in turn restores blood pressure to normal. Accordingly, during sepsis, the widespread activation of the sympathetic nervous system is thought to be the response to the developing hypotension. However, Vayssettes-Courchay et al. found that sympathetic activation and tachycardia in sepsis were unrelated to the baroreflex. They demonstrated that LPS induced a fall in blood pressure and an increase in heart rate and renal sympathetic nerve activity. Baroreceptor and chemoreceptor denervation accelerated the reduction in blood pressure but slightly pronounced renal sympathetic activation. Electrolytic lesioning of the nucleus tractus solitarius or blocking the effects of baroreflex efferents by α2-adrenoceptor antagonist failed to alter the

effects of LPS on renal sympathetic nerve activity and heart rate in rats. These results indicate that the sympathetic nerve activation, which triggers tachycardia, is not dependent on the baroreflex pathway and the hypotension during sepsis [34]. Recently, Booth et al. found that intracarotid infusion of prostaglandin E_2 in conscious sheep increased cardiac sympathetic nerve activity and heart rate, but not renal sympathetic nerve activity or mean arterial pressure. Treatment with indomethacin, a nonselective cyclooxygenase inhibitor, 8 h after sepsis insult reduced cardiac sympathetic nerve activity and heart rate without altering baroreflex control. These results demonstrated that the control of sympathetic outflow to the heart and kidneys is different and central prostaglandin E_2 may contribute to the increases in cardiac sympathetic nerve activity and heart rate during sepsis [35]. Furthermore, intravenous administration of immunosuppressant drug cyclosporine A reversed the LPS-induced tachycardia and decreases in HRV in rats; these effects of cyclosporine A disappeared after intracisternal injection of soluble guanylate cyclase inhibitor. The amelioration by cyclosporine A of LPS-induced reductions in HRV was also abolished in the presence of JNK inhibitor or ERK inhibitor. These findings suggest that cyclosporine counteracts LPS-evoked decrease in cardiac sympathetic dysfunction via central soluble guanylate cyclase and MAPK signal pathways in rats. In addition, the treatment with JNK inhibitor or ERK inhibitor not only reversed the cyclosporine A-evoked increases in HRV but also resulted in further decreases in HRV in LPS-treated rats, indicating the importance of central MAPK signaling in the cardiac autonomic dysfunction during sepsis [36].

On the other hand, a reduction in vagal tone has also been shown in anesthetized endotoxic rabbits [37]. Compared to sham rats, the isolated septic rat hearts had reduced levels of choline in their right atriums, whereas norepinephrine concentration in blood plasma was increased. These results demonstrate that cardiac autonomic nervous dysfunction includes sympathetic overexcitation and parasympathetic suppression [38]. Mazloom et al. observed the role of alpha7 nicotinic acetylcholine receptors (α7nACHR) in modulation of heart rate dynamics in LPS-induced septic rats and found that α7nACHR is expressed in the rat atrium and mainly localized at the endothelial layer. Systemic administration of an α7nACHR antagonist did not affect heart rate dynamics in control rats. In contrast, α7nACHR blockade further reduced HRV in LPS-treated rats. In addition, α7nACHR agonist was unable to regulate heart rate dynamics in LPS-challenged rats, suggesting a tonic role for α7nACHR of acetylcholine in modulation of heart rate dynamics during sepsis [39].

Cardiac autonomic dysfunction results not only from the autonomic nervous system dysfunction but also from the impairment in the signal transduction pathways and/or ion channels mediating the autonomic nervous signals in the heart itself. Binding of the sympathetic neurotransmitter, norepinephrine, to cardiac adrenergic receptors (ARs) and parasympathetic neurotransmitter, acetylcholine, to muscarinic receptors triggers signal transduction pathways and finally results in a modulation of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel-mediated I_f current in the cardiac pacemaker cells. LPS was found to interact with cardiac HCN ion channels and sensitized cardiac HCN ion channels to sympathetic signals, which directly inhibited cardiac I_f current; this affected transmitting sympathetic

and vagal signals on the heart. These findings suggest that the impairment in cardiac HCN ion channel-mediated signal transduction pathways might also contribute to the reduced HRV in patients with sepsis [40].

6.3.2 Effects of Alpha-Adrenergic Signaling on SICD

The sympathetic neurotransmitter norepinephrine exerts its effects via binding to G-protein-coupled α- and β-ARs. α-ARs include α_1 and α_2 subtypes. Generally, norepinephrine has the highest affinity for α_2-ARs, a high norepinephrine concentration acts on both α_2-ARs and α_1-ARs, whereas at low concentrations, norepinephrine predominantly stimulates α_2-ARs [41]. In the rodent and human heart, α_{1A}- and α_{1B}-ARs are expressed in cardiac myocytes, whereas the α_{1D} is observed in coronary smooth muscle cells. Indeed, α_1-AR expression is maintained or even increased in the process of heart failure. A large number of investigations demonstrated that myocardial α_1-ARs exert adaptive and protective effects in the setting of heart failure, including protection against cardiomyocyte death, increased glucose metabolism, enhanced protein synthesis, and positive inotropy. α_1-AR activation might attenuate the toxic effects of β-ARs in the chronic heart failure. Clinical investigations have demonstrated that blockade of α_1-ARs is associated with incident heart failure in hypertensive patients. These results suggest that activation of α_1-ARs might represent a novel therapeutic approach to the treatment of heart failure [42]. However, data are scarce regarding the myocardial effects of α_1-AR agonist during sepsis. In 1991, Gregory et al. observed that administration of phenylephrine, a selective α_1-AR agonist, significantly increased mean arterial pressure, systemic vascular resistance index, left ventricular stroke work index, and stroke volume index, accompanied by the decreased blood lactate concentrations and increased urine output in patients with septic shock [43]. In contrast, animal experimental study showed that norepinephrine administered 18 h after septic challenge improved mean arterial pressure and preload recruitable stroke work (a load-independent measure of systolic function) as well as diastolic function and ventriculoarterial coupling, whereas phenylephrine, an α_1-AR agonist, exhibited deleterious effects on systolic and diastolic function as well as ventriculoarterial coupling in septic rats induced by peritonitis [44]. Therefore, norepinephrine may be a more reliable strategy as a first-line therapy during septic shock. Recently, our laboratory demonstrated that norepinephrine inhibited LPS-induced TNF-α production in an α_1-AR-dependent manner. In cultured neonatal rat cardiomyocytes, norepinephrine also suppressed p38 phosphorylation and NF-κB activation and promoted extracellular signal-regulated kinase 1/2 (ERK1/ERK2) phosphorylation and c-Fos expression in LPS-challenged cardiomyocytes, all of which were blocked by α_1-AR antagonist pretreatment. Furthermore, treatment with U0126, a selective ERK1/ERK2 inhibitor, reversed the effects of norepinephrine on c-Fos expression, p38 phosphorylation, and TNF-α production, but not NF-κB activation in LPS-treated cardiomyocytes. In addition, in endotoxemic mice, phenylephrine increased myocardial ERK1/ERK2 phosphorylation and c-Fos expression, inhibited p38 and

NF-κB activation, and attenuated myocardial TNF-α production and cardiac dysfunction. These findings suggest that α_1-AR activation by norepinephrine attenuates cardiomyocyte TNF-α production and then cardiac dysfunction during endotoxemia through promoting myocardial ERK activation as well as suppressing NF-κB activation [45]. Accordingly, more research is needed to investigate the effects of myocardial α_1-AR activation on sepsis-induced cardiac dysfunction.

On the other hand, a specific antagonist for α2A-AR, BRL-44408 maleate, was found to reduce the serum levels of proinflammatory cytokines, multiple organ dysfunction, and the mortality rate in CLP-induced septic rats [46]. We have recently demonstrated that α2-AR antagonist, yohimbine, attenuates LPS-induced cardiac dysfunction. Yohimbine reduced cardiac α2A-AR level and promoted cardiac norepinephrine release in LPS-treated mice. Reserpine that exhausted cardiac norepinephrine without markedly decreasing plasma norepinephrine level abolished the inhibitory effects of yohimbine on cardiac TNF-α and iNOS expression as well as cardiac dysfunction, but not the suppressive effect of yohimbine on plasma TNF-α in endotoxemic mice. Furthermore, both yohimbine and reserpine significantly inhibited LPS-induced myocardial apoptosis. α_1-AR, β_2-AR, but not β_1-AR antagonists abrogated the inhibitory effect of yohimbine on LPS-stimulated myocardial apoptosis. However, β_1-AR antagonist attenuated LPS-evoked cardiomyocyte apoptosis, partly reversed the protective effect of yohimbine on the left ventricular EF in LPS-challenged mice. These results indicate that yohimbine inhibits LPS-induced cardiac dysfunction, at least in part, through blocking sympathetic presynaptic α_{2A}-AR and promoting cardiac norepinephrine release. The increased concentrations of cardiac norepinephrine improve cardiac function via inhibiting myocardial iNOS and TNF-α expression, activating β1-AR as well as reducing cardiomyocyte apoptosis via activating α_1-AR and β_2-AR in LPS-treated mice. Thus, the inhibition of α_{2A}-AR may provide a novel therapeutic strategy for cardiac dysfunction in septic patients [47].

6.3.3 Effects of Beta-Adrenergic Signaling on SICD

The role of β-AR activation in sepsis-induced cardiac dysfunction has been investigated for several decades. In patients with septic shock, impaired β-AR activation of cyclic adenosine monophosphate is associated with myocardial hyporesponsiveness to catecholamines, indicating that β-AR desensitization may contribute to the sepsis-induced cardiac dysfunction [48]. Administration of milrinone, a selective phosphodiesterase inhibitor that has inotropic effects via increasing intracellular cyclic adenosine monophosphate levels in cardiomyocytes, and β-AR agonist, dobutamine, significantly improved systolic function in LPS-injected rabbits [49]. In pediatric patients with septic shock, milrinone also significantly increased cardiac index, stroke volume index, and right and left ventricular stroke work index [50]. Nowadays, β_1-AR agonist dobutamine is recommended as an inotrope for septic patients with persistent hypoperfusion despite adequate fluid loading and the use of vasopressor agents [51]. However, the usefulness of dobutamine to treat cardiac

dysfunction in septic shock is limited. It was observed that the effect of dobutamine as a positive inotrope is impaired in sepsis due to cyclic adenosine monophosphate breakdown resulted from upregulated phosphodiesterase 4 [52]. In particular, we recently demonstrated that exhausted cardiac endogenous norepinephrine or blockade of β_1-AR almost completely abrogated cardiomyocyte apoptosis in LPS-treated mice and β_1-AR activation might be more important than cytokines in LPS-evoked cardiomyocyte apoptosis [47]. β_1-AR stimulation promoted LPS-induced cardiomyocyte apoptosis via activating protein kinase A and increasing the phosphorylation Ca^{2+}/calmodulin-dependent protein kinase II and IκB [53]. Conversely, peripheral β_1-AR blockade exerted anti-inflammatory and cardioprotective effects, with mortality decrease if administered before a septic insult [54]. A monocentric randomized clinical trial showed that treatment with esmolol, a short-time acting β1-AR antagonist, titrated to reduce heart rate below 95 beats/min over a 96-h period, significantly increased the left ventricular stroke work index, and decreased arterial blood lactate level and 28-day mortality rate, without increased adverse events, in patients with septic shock [55]. But despite all that, there is insufficient evidence to justify the routine use of β-blockers in sepsis to date; further large multicenter randomized clinical trials are needed to confirm the potential benefit of β-blockers in patients with severe sepsis [56]. In addition, β-blockers have been found to increase vagal activity and reduce sympathetic tone, which restore the sympathetic-vagal balance. Treatment with β-blockers has been proven to increase HRV in patients with diabetes, coronary artery disease, chronic heart failure, or multiple organ dysfunction syndrome [40, 57], but it is unclear whether administration of β_1-AR antagonist increases HRV in septic patients.

In adult rat ventricular cardiomyocytes, β_1-AR stimulation increases apoptosis in a cAMP-dependent manner, whereas stimulation of β_2-AR inhibits apoptosis by a G(i)-coupled pathway [58]. Some studies suggest that β_2-AR stimulating agents may represent the preferred therapy for inflammatory myocardial disease [59]. However, at present, there is no study regarding effects of β_2-AR stimulating agents on sepsis-induced cardiac dysfunction. Although the previous study showed that β_3-AR mediated an increased negative inotropic response to its agonists and was upregulated in the human and murine myocardium, indicating the role of β_3-AR activation in the pathogenesis of sepsis-induced cardiac dysfunction [60], very little research focused on the immunoregulatory effect of β_3-AR activation on the heart during sepsis.

6.3.4 Effect of Cholinergic Signaling on SICD

In 2000, Tracey et al. observed that direct electrical stimulation of the peripheral efferent vagus nerve significantly inhibited TNF production in the liver, attenuated serum TNF concentration, and prevented the development of shock in LPS-challenged rats. In vitro experiments showed that acetylcholine (ACh), the principal vagal neurotransmitter, significantly decreased the release of cytokines, such as TNF-α, IL-1β, and IL-6, in LPS-treated human macrophages [61]. Accordingly, the concept of cholinergic anti-inflammatory pathway was first proposed [62]. Many

studies have demonstrated that this anti-inflammatory pathway requires α7nAchRs expressed on macrophages, neurons, and other cells. Ach stimulates α7nAchRs in inflammatory cells, in turn attenuating the production and release of proinflammatory cytokines via activating the Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling and suppressing the NF-κB pathway [59]. Recently, the effect of vagal stimulation on myocardial function and inflammation was observed in vagotomized, endotoxemic rats. Compared with endotoxemic rats with an intact vagus nerve, the survival time was reduced in endotoxemic rats with right cervical vagotomy; the right cervical vagotomy aggravated LPS-induced cardiac dysfunction, with further elevated cytokine levels of TNF-α and IL-1β in the ventricular tissues, all of which were reversed by the stimulation of the right cervical vagus nerve. These studies suggest that vagal stimulation may be an important therapeutic strategy in the treatment of septic patients with cardiac dysfunction [63]. Despite the fact that ventricular myocardium is only sparsely innervated by the vagus nerve and a strong innervation takes place at the sinoatrial node, immunohistochemistry demonstrated α7 subunits of nAChR on cardiac neurons, fibroblasts, and cardiomyocytes [64], including cultured primary neonatal rat ventricular cardiomyocytes and a human cardiomyocyte cell line [65]. Furthermore, cardiomyocytes were found to possess an ACh synthesis system, which is positively modulated by cholinergic stimuli. Such an amplification system in cardiomyocytes may contribute to the beneficial effects of vagal stimulation on the ventricles [66]. Thus, vagal stimulation-induced cardioprotective effect may be associated with cardiomyocyte α7nAchRs. Although recent study observed that ACh pretreatment ameliorated H_2O_2-induced intracellular Ca^{2+} dyshomeostasis via stimulating both muscarinic and nicotinic receptors in isolated rat cardiomyocytes [67], limited data are available concerning the effect of α7nAchR activation on cardiomyocytes in sepsis.

6.4 Conclusions and Future Perspective

Although advances in understanding of the mechanisms of sepsis-induced cardiac dysfunction have been gained during recent years, no specific therapeutic strategies have been defined as yet. Sepsis-induced cardiac dysfunction is the result of the interplay between inflammation and cardiac autonomic nervous system. In sepsis, the cardiac autonomic nervous system imbalance is characterized by marked sympathetic activation and parasympathetic suppression. A large amount of evidence over the past decades has proved the immunomodulatory effect of the autonomic nervous system on the heart in sepsis. The discovery of the cholinergic anti-inflammatory pathway as well as the actions of α_{2A}-AR and β-AR blockade has paved the way for new therapeutic approaches to treat sepsis-induced cardiac dysfunction. Further understanding of the effects of autonomic nervous system regulation on cardiomyocytes, endothelial cells, and fibroblasts of the heart during sepsis will expand our knowledge of mechanisms for sepsis-induced cardiac dysfunction. This will help identify the new cardiac-specific therapeutic strategy and improve the outcome in septic patients.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–10.
2. Esposito S, De Simone G, Boccia G, De Caro F, Pagliano P. Sepsis and septic shock: new definitions, new diagnostic and therapeutic approaches. J Glob Antimicrob Resist. 2017;10:204–12.
3. Rhee C, Dantes R, Epstein L, Murphy DJ, Seymour CW, Iwashyna TJ, et al. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. JAMA. 2017;318(13):1241–9.
4. Driessen RGH, van de Poll MCG, Mol MF, van Mook WNKA, Schnabel RM. The influence of a change in septic shock definitions on intensive care epidemiology and outcome: comparison of sepsis-2 and sepsis-3 definitions. Infect Dis (Lond). 2017;26:1–7.
5. Vallabhajosyula S, Kumar M, Pandompatam G, Sakhuja A, Kashyap R, Kashani K, Gajic O, Geske JB, Jentzer JC. Prognostic impact of isolated right ventricular dysfunction in sepsis and septic shock: an 8-year historical cohort study. Ann Intensive Care. 2017;7(1):94.
6. Hochstadt A, Meroz Y, Landesberg G. Myocardial dysfunction in severe sepsis and septic shock: more questions than answers? J Cardiothorac Vasc Anesth. 2011;25(3):526–35.
7. Palmieri V, Innocenti F, Guzzo A, Guerrini E, Vignaroli D, Pini R. Left ventricular systolic longitudinal function as predictor of outcome in patients with sepsis. Circ Cardiovasc Imaging. 2015;8(11):e003865.
8. Sanfilippo F, Corredor C, Arcadipane A, Landesberg G, Vieillard-Baron A, Cecconi M, Fletcher N. Tissue Doppler assessment of diastolic function and relationship with mortality in critically ill septic patients: a systematic review and meta-analysis. Br J Anaesth. 2017;119(4):583–94.
9. de Castilho FM, Ribeiro ALP, da Silva JLP, Nobre V, de Sousa MR. Heart rate variability as predictor of mortality in sepsis: a prospective cohort study. PLoS One. 2017;12(6):e0180060.
10. Rudiger A, Dyson A, Felsmann K, Carré JE, Taylor V, Hughes S, et al. Early functional and transcriptomic changes in the myocardium predict outcome in a long-term rat model of sepsis. Clin Sci (Lond). 2013;124:391–401.
11. Li C, Hua F, Ha T, Singh K, Lu C, Kalbfleisch J, et al. Activation of myocardial phosphoinositide-3-kinase p110α ameliorates cardiac dysfunction and improves survival in polymicrobial sepsis. PLoS One. 2012;7:e44712.
12. Antonucci E, Fiaccadori E, Donadello K, Taccone FS, Franchi F, Scolletta S. Myocardial depression in sepsis: from pathogenesis to clinical manifestations and treatment. J Crit Care. 2014;29:500–11.
13. Zaky A, Deem S, Bendjelid K, Treggiari MM. Characterization of cardiac dysfunction in sepsis: an ongoing challenge. Shock. 2014;41:12–24.
14. Calvin JE, Driedger AA, Sibbald WJ. An assessment of myocardial function in human sepsis utilizing ECG gated cardiac scintigraphy. Chest. 1981;80:579–86.
15. Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, Damske BA, Parrillo JE. Profound but reversible myocardial depression in patients with septic shock. Ann Intern Med. 1984;100:483–90.
16. Romero-Bermejo FJ, Ruiz-Bailen M, Gil-Cebrian J, Huertos-Ranchal MJ. Sepsis-induced cardiomyopathy. Curr Cardiol Rev. 2011;7:163–83.
17. Sato R, Kuriyama A, Takada T, Nasu M, Luthe SK. Prevalence and risk factors of sepsis-induced cardiomyopathy: a retrospective cohort study. Medicine (Baltimore). 2016;95:e5031.
18. Huang SJ, Nalos M, McLean AS. Is early ventricular dysfunction or dilatation associated with lower mortality rate in adult severe sepsis and septic shock? A meta-analysis. Crit Care. 2013;17(3):R96.
19. Burns AT, La Gerche A, D'hooge J, MacIsaac AI, Prior DL. Left ventricular strain and strain rate: characterization of the effect of load in human subjects. Eur J Echocardiogr. 2010;11(3):283–9.

20. Dalla K, Hallman C, Bech-Hanssen O, Haney M, Ricksten SE. Strain echocardiography identifies impaired longitudinal systolic function in patients with septic shock and preserved ejection fraction. Cardiovasc Ultrasound. 2015;13:30.
21. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2016;17(12):1321–60.
22. Sanfilippo F, Corredor C, Fletcher N, Landesberg G, Benedetto U, Foex P, Cecconi M. Diastolic dysfunction and mortality in septic patients: a systematic review and meta-analysis. Intensive Care Med. 2015;41:1004–13.
23. Tateishi Y, Oda S, Nakamura M, Watanabe K, Kuwaki T, Moriguchi T, Hirasawa H. Depressed heart rate variability is associated with high IL-6 blood level and decline in the blood pressure in septic patients. Shock. 2007;28:549–53.
24. de Castilho FM, Ribeiro ALP, da Silva JLP, Nobre V, de Sousa MR. Heart rate variability as predictor of mortality in sepsis: a prospective cohort study. PLoS One. 2017;12:e0180060.
25. Pinto BB, Ritter C, Michels M, Gambarotta N, Ferrario M, Dal-Pizzol F, Singer M. Characterization of brain-heart interactions in a rodent model of sepsis. Mol Neurobiol. 2017;54:3745–52.
26. Bohanon FJ, Mrazek AA, Shabana MT, Mims S, Radhakrishnan GL, Kramer GC, Radhakrishnan RS. Heart rate variability analysis is more sensitive at identifying neonatal sepsis than conventional vital signs. Am J Surg. 2015;210:661–7.
27. Kobayashi M, Massiello A, Karimov JH, Van Wagoner DR, Fukamachi K. Cardiac autonomic nerve stimulation in the treatment of heart failure. Ann Thorac Surg. 2013;96:339–45.
28. Li CY, Li YG. Cardiac sympathetic nerve sprouting and susceptibility to ventricular arrhythmias after myocardial infarction. Cardiol Res Pract. 2015;2015:698368.
29. Rudiger A, Singer M. The heart in sepsis: from basic mechanisms to clinical management. Curr Vasc Pharmacol. 2013;11:187–95.
30. Liu YC, Yu MM, Shou ST, Chai YF. Sepsis-induced cardiomyopathy: mechanisms and treatments. Front Immunol. 2017;8:1021.
31. Lv X, Wang H. Pathophysiology of sepsis-induced myocardial dysfunction. Mil Med Res. 2016;3:30.
32. Sharshar T, Gray F, Lorin de la Grandmaison G, Hopkinson NS, Ross E, Dorandeu A, Orlikowski D, Raphael JC, et al. Apoptosis of neurons in cardiovascular autonomic centres triggered by inducible nitric oxide synthase after death from septic shock. Lancet. 2003;362:1799–805.
33. Ramchandra R, Wan L, Hood SG, Frithiof R, Bellomo R, May CN. Septic shock induces distinct changes in sympathetic nerve activity to the heart and kidney in conscious sheep. Am J Physiol Regul Integr Comp Physiol. 2009;297:R1247–53.
34. Vayssettes-Courchay C, Bouysset F, Verbeuren TJ. Sympathetic activation and tachycardia in lipopolysaccharide treated rats are temporally correlated and unrelated to the baroreflex. Auton Neurosci. 2005;120:35–45.
35. Booth LC, Ramchandra R, Calzavacca P, May CN. Role of prostaglandins in determining the increased cardiac sympathetic nerve activity in ovine sepsis. Am J Physiol Regul Integr Comp Physiol. 2014;307:R75–81.
36. Sallam MY, El-Gowilly SM, Abdel-Galil AA, El-Mas MM. Cyclosporine counteracts endotoxemia-evoked reductions in blood pressure and cardiac autonomic dysfunction via central sGC/MAPKs signaling in rats. Eur J Pharmacol. 2017;797:143–52.
37. Tang CH, Chan GS, Middleton PM, Cave G, Harvey M, Savkin AV, Lovell NH. Transfer function analysis of baroreflex function in a rabbit model of endotoxic shock. Conf Proc IEEE Eng Med Biol Soc. 2009;2009:1848–51.
38. Contreras P, Migliaro ER, Suhr B. Right atrium cholinergic deficit in septic rats. Auton Neurosci. 2014;180:17–23.
39. Mazloom R, Eftekhari G, Rahimi-Balaei M, Khori V, Hajizadeh S, Dehpour AR, Mani AR. The role of α7 nicotinic acetylcholine receptor in modulation of heart rate dynamics in endotoxemic rats. PLoS One. 2013;8(12):e82251.

40. Werdan K, Schmidt H, Ebelt H, Zorn-Pauly K, Koidl B, Hoke RS, Heinroth K, Müller-Werdan U. Impaired regulation of cardiac function in sepsis, SIRS, and MODS. Can J Physiol Pharmacol. 2009;87(4):266–74.
41. Maletic V, Eramo A, Gwin K, Offord SJ, Duffy RA. The role of norepinephrine and its α-adrenergic receptors in the pathophysiology and treatment of major depressive disorder and schizophrenia: a systematic review. Front Psych. 2017;8:42.
42. Jensen BC, O'Connell TD, Simpson PC. Alpha-1-adrenergic receptors in heart failure: the adaptive arm of the cardiac response to chronic catecholamine stimulation. J Cardiovasc Pharmacol. 2014;63:291–301.
43. Gregory JS, Bonfiglio MF, Dasta JF, Reilley TE, Townsend MC, Flancbaum L. Experience with phenylephrine as a component of the pharmacologic support of septic shock. Crit Care Med. 1991;19:1395–400.
44. Ducrocq N, Kimmoun A, Furmaniuk A, Hekalo Z, Maskali F, Poussier S, Marie PY, Levy B. Comparison of equipressor doses of norepinephrine, epinephrine, and phenylephrine on septic myocardial dysfunction. Anesthesiology. 2012;116:1083–91.
45. Yu X, Jia B, Wang F, Lv X, Peng X, Wang Y, Li H, Wang Y, Lu D, Wang H. α1 adrenoceptor activation by norepinephrine inhibits LPS-induced cardiomyocyte TNF-α production via modulating ERK1/2 and NF-κB pathway. J Cell Mol Med. 2014;18:263–73.
46. Zhang F, Wu R, Qiang X, Zhou M, Wang P. Antagonism of alpha2A-adrenoceptor: a novel approach to inhibit inflammatory responses in sepsis. J Mol Med (Berl). 2010;88(3):289–96.
47. Wang Y, Yu X, Wang F, Wang Y, Wang Y, Li H, Lv X, Lu D, Wang H. Yohimbine promotes cardiac NE release and prevents LPS-induced cardiac dysfunction via blockade of presynaptic α2A-adrenergic receptor. PLoS One. 2013;8:e63622.
48. Silverman HJ, Penaranda R, Orens JB, Lee NH. Impaired beta-adrenergic receptor stimulation of cyclic adenosine monophosphate in human septic shock: association with myocardial hyporesponsiveness to catecholamines. Crit Care Med. 1993;21:31–9.
49. Barraud D, Faivre V, Damy T, Welschbillig S, Gayat E, Heymes C, Payen D, Shah AM, Mebazaa A. Levosimendan restores both systolic and diastolic cardiac performance in lipopolysaccharide-treated rabbits: comparison with dobutamine and milrinone. Crit Care Med. 2007;35:1376–82.
50. Barton P, Garcia J, Kouatli A, Kitchen L, Zorka A, Lindsay C, Lawless S, Giroir B. Hemodynamic effects of i.v. milrinone lactate in pediatric patients with septic shock. A prospective, double-blinded, randomized, placebo-controlled, interventional study. Chest. 1996;109:1302–12.
51. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Intensive Care Med. 2017;43(3):304–77.
52. Sakai M, Suzuki T, Tomita K, Yamashita S, Palikhe S, Hattori K, Yoshimura N, Matsuda N, Hattori Y. Diminished responsiveness to dobutamine as an inotrope in mice with cecal ligation and puncture-induced sepsis: attribution to phosphodiesterase 4 upregulation. Am J Physiol Heart Circ Physiol. 2017;312:H1224–37.
53. Wang Y, Wang Y, Yang D, Yu X, Li H, Lv X, Lu D, Wang H. β_1-adrenoceptor stimulation promotes LPS-induced cardiomyocyte apoptosis through activating PKA and enhancing CaMKII and IκBα phosphorylation. Crit Care. 2015;19:76.
54. Ackland GL, Yao ST, Rudiger A, Dyson A, Stidwill R, Poputnikov D, Singer M, Gourine AV. Cardioprotection, attenuated systemic inflammation, and survival benefit of beta1-adrenoceptor blockade in severe sepsis in rats. Crit Care Med. 2010;38(2):388–94.
55. Morelli A, Ertmer C, Westphal M, Rehberg S, Kampmeier T, Ligges S, et al. Effect of heart rate control with esmolol on hemodynamic and clinical outcomes in patients with septic shock: a randomized clinical trial. JAMA. 2013;310:1683–91.
56. Chacko CJ, Gopal S. Systematic review of use of β-blockers in sepsis. J Anaesthesiol Clin Pharmacol. 2015;31:460–5.

57. Hennen R, Friedrich I, Hoyer D, Nuding S, Rauchhaus M, Schulze M, et al. Autonomic dysfunction and beta-adrenergic blockers in multiple organ dysfunction syndrome. Dtsch Med Wochenschr. 2008;133:2500–4.
58. Communal C, Singh K, Sawyer DB, Colucci WS. Opposing effects of beta(1)- and beta(2)-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. Circulation. 1999;100:2210–2.
59. Cheng Z, Li-Sha G, Yue-Chun L. Autonomic nervous system in viral myocarditis: pathophysiology and therapy. Curr Pharm Des. 2016;22:485–98.
60. Moniotte S, Belge C, Sekkali B, Massion PB, Rozec B, Dessy C, Balligand JL. Sepsis is associated with an upregulation of functional beta3 adrenoceptors in the myocardium. Eur J Heart Fail. 2007;9:1163–71.
61. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405:458–62.
62. Tracey KJ. The inflammatory reflex. Nature. 2002;420(6917):853–9.
63. Schulte A, Lichtenstern C, Henrich M, Weigand MA, Uhle F. Loss of vagal tone aggravates systemic inflammation and cardiac impairment in endotoxemic rats. J Surg Res. 2014;188:480–8.
64. Dvorakova M, Lips KS, Brüggmann D, Slavikova J, Kuncova J, Kummer W. Developmental changes in the expression of nicotinic acetylcholine receptor alpha-subunits in the rat heart. Cell Tissue Res. 2005;319:201–9.
65. Mavropoulos SA, Khan NS, Levy ACJ, Faliks BT, Sison CP, Pavlov VA, Zhang Y, Ojamaa K. Nicotinic acetylcholine receptor-mediated protection of the rat heart exposed to ischemia reperfusion. Mol Med. 2017;23:120.
66. Kakinuma Y, Akiyama T, Sato T. Cholinoceptive and cholinergic properties of cardiomyocytes involving an amplification mechanism for vagal efferent effects in sparsely innervated ventricular myocardium. FEBS J. 2009;276:5111–25.
67. Palee S, Apaijai N, Shinlapawittayatorn K, Chattipakorn SC, Chattipakorn N. Acetylcholine attenuates hydrogen peroxide-induced intracellular calcium dyshomeostasis through both muscarinic and nicotinic receptors in cardiomyocytes. Cell Physiol Biochem. 2016;39:341–9.

Role of TLR4 in Sepsis

7

Jing Tang

7.1 The Definition and the Status Quo of Sepsis

Sepsis, a syndrome of physiologic pathologic and biochemical abnormalities induced by infection, is a major public health concern. The concept of sepsis changed as time goes by. For the Ancient Greeks, sepsis referred to rot decay or putrefaction. The modern concept of sepsis has focused on the human response to invading organisms [1]. According to the Third International Consensus Definitions for Sepsis and Septic Shock, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Moreover, patients with sepsis and evidence of organ dysfunction including cardiovascular renal hepatic or neurological were classified as having "severe sepsis." Meanwhile, septic shock is a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality [2–4].

Though in parallel to the advancement of health care, the number of patients suffering from sepsis has been on rise. It accounts for 1,000,000 cases and 200,000 deaths annually in the United States alone. In England, the proportion and numbers of sepsis admissions increased from 23.5% in 2000 to 25.2% in 2012 and was recently reported to be still rising [5–8]. Moreover, of these patients, half are treated in the intensive care unit (ICU). Studies from other high-income countries show similar rates of sepsis in the ICU. The incidence of sepsis outside modern ICUs, especially in parts of the world in which ICU care is scarce, is largely unknown [9].

The timely and accurate diagnosis of sepsis is difficult because the onset of sepsis is always latent and the response that predominates in the clinical phenotype varies across patients [1]. The clinical manifestations of sepsis are highly variable depending on the initial site of infection, the causative organism, the pattern of acute organ dysfunction, the underlying health status of the patient, and the interval before initiation of treatment

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[9]. In most cases, sepsis occurs in patients with disabilities or illnesses mentioned above, without regard to race, geography, or age. The general characteristics may include fever, tachycardia, and tachypnea, followed by mental confusion, transient hypotension, diminished urine output, or unexplained thrombocytopenia [3, 10]. If not treated timely and appropriately, the patient may develop respiratory or renal failure, central nervous system dysfunction, disseminated intravascular coagulation, and unresponsive hypotension [3]. Fortunately, with advances in training, better surveillance and monitoring, and prompt initiation of therapy to treat the underlying infection and support failing organs, the mortality is failing as closer to 20–30% in many series.

Focus on the existing data, we may say that sepsis represents a failure of homeostasis that results from dysfunction of the neuroendocrine and immune systems, which responds to infection and injury. The immuno- and neurophysiological mechanisms of cellular and organ dysfunction in sepsis, however, are poorly understood. As a disease with high mortality, the ultimate cause of death in patients is multiple organ failure. Unfortunately, the pathogenesis of organ dysfunction is multifactorial and incompletely understood as well.

Over the past years, researchers and clinical workers are working hard in finding the cure for sepsis, but all failed with considerable frustration. Unlike other major epidemic illnesses, treatment for sepsis is nonspecific. The Surviving Sepsis Campaign (SSC) has developed guidelines for the treatment of sepsis and septic shock. The first 60 min after the recognition of shock is considered the "golden hour" during which time therapies need to be initiated to reverse the shock. First of all, early effective fluid resuscitation is crucial for stabilization of sepsis-induced tissue hypoperfusion or septic shock, which is known as early goal-directed therapy (EGDT), and the goals during the first 6 h of resuscitation include a central venous pressure 8–12 mmHg, a mean arterial pressure (MAP) ≥65 mmHg, a urine output ≥0.5 mL/kg/h, and a superior vena cava venous oxygen saturation of ≥70% [11]. Then, rapid administration of appropriate and adequate antimicrobials is of great essentiality. Antimicrobials administered within the first hour of recognition of sepsis and septic shock should be the "goal" of therapy and target all likely pathogens. The third is source control, including drainage of an abscess, debridement of infected necrotic tissue, removal of a potentially infected device, and definitive control of a source of ongoing microbial contamination. The fourth is the use of vasoactive medications, such as norepinephrine, epinephrine, dopamine, and dobutamine. All these vasopressor therapies are used to meet the target of a mean arterial pressure of ≥65 mmHg to maintain an adequate tissue perfusion. Other supportive therapies of sepsis include steroid therapy for refractory septic shock, the transfusion of blood products when it is necessary, continuous renal replacement therapy, oxygen therapy, and mechanical ventilation [12].

However, the treatment strategies above are not always able to achieve the ideal clinical efficacy; one practical problem is that patients often come to the hospital relatively late in the disease. In the first place, acute response to invading pathogens induces a cytokine storm characterized by high plasma levels of pro-inflammatory cytokines, and blocking these early cytokines may simply be too late [3]. The immunopathology that responds to sepsis and the pathophysiology of sepsis are both not fully understood at present; further research into sepsis need to be ferreted out to provide better treatment strategies [10, 13].

7.2 The Role of TLR Family, Especially TLR4, in Sepsis and Its Signaling Pathway and Significance

Here we will review the mechanisms and treatment progress of TLR4, focusing on their signaling pathways and the possible therapeutic targets.

Toll-like receptors (TLRs), as one kind of pattern recognition receptors (PRRs) [14], which are detected by the innate immune system as a result of tissue injury or inflammation through various innate immune receptors, can recognize distinct microbial components and directly activate immune cells, for instance, macrophages, dendritic cells (DCs), B cells, specific types of T cells, and even nonimmune cells such as fibroblasts and epithelial cells [3, 15]. Exposure of immune cells to the ligands of these receptors activates intracellular signaling cascades that rapidly induce the expression of a variety of overlapping and unique genes involved in the inflammatory and immune responses.

The TLR family was first discovered as a gene product essential for determining the dorsal-ventral patterning during embryogenesis in *Drosophila* [16]. To date, 13 members of the TLR family have been identified in mammals. Of them, 10 TLRs are expressed in human macrophages and mice express all the 13 TLRs [17].TLRs may be expressed extra- or intracellularly. Meanwhile certain TLRs (TLRs 1, 2, 4, 5, 6, and 10) are expressed on the cell surface, others (TLRs 3, 7, 8, and 9) are found almost exclusively in intracellular compartments such as endosomes, and their ligands, mainly nucleic acids, require internalization to the endosome before signaling is possible. The transmembrane and membrane-proximal regions are important for the cellular compartmentalization of these receptors. However, TLR4 is expressed on and signals from both the cell surface and the endosomal membrane. Furthermore, each TLR has their specific ligands. For example, TLR2 interacts with TLR1 and TLR6, recognizing various bacterial components, such as peptidoglycan, lipo-peptide and lipoprotein of Gram-positive bacteria, and mycoplasma lipopeptide [15, 18, 19]. Double-stranded RNA, which is produced from diverse viruses during replication, is recognized by TLR3 [15]. TLR5's PAMP is flagellin [15, 20]. TLR7 and TLR8 genes show high homology to each other, for example, mice TLR7 and human TLR8 recognizing synthetic antiviral imidazoquinoline components and some guanine nucleotide analogs as well as uridine-rich or uridine/guanosine-rich ssRNA [21, 22]. TLR9 recognizes bacterial and viral CaG DNA motifs and malaria pigment hemozoin and mediates IFN-α response to some virus. Herpes simplex virus type-1 induces IFN-alpha production via Toll-like receptor 9-dependent and Toll-like receptor 9-independent pathways. And the murine TLR11 recognized a soluble extract of *T. gondii* tachyzoites (STAg), while human TLR11 is nonfunctional due to the presence of a stop codon in the gene [15, 23] (Fig. 7.1). And TLR4 recognizes most of the Gram-negative bacteria by the component on their outer membranes, lipopolysaccharide (LPS), which plays a vital role in the sepsis and septic shock.

The innate immune response is the first line of defense against microbial pathogens. Innate immune system relies on a limited number of germline-encoded receptors; these receptors evolved to recognize conserved products of microbial

Triacyl lipopeptide (bacteria, mycoplasma)	Peptidoglycan (gram-positive bacteria) Lipoarabinomannan (mycobacteria)	Diacyl lipopeptide (mycoplasma)		LPS (gram-negative bacteria)	Flagellin		CpG DNA	Uropathogenic bacteria components
			dsRNA	Envelope proteins (RSV, MMTV)		ssRNA	CpG DNA	
							Hemozoin (plasmodium)	Profilin-like molecules (toxoplasma gongii)
						Antiviral imidazoquinoline, guanine nucleotide analogs,uridine-rich or uridine/guanosine-rich ssRNA		
TLR2/1	TLR2	TLR2/6	TLR3	TLR4	TLR5	TLR7, TLR8	TLR9	TLR11

Fig. 7.1 TLRs, TLR ligands, and TIR-domain-containing adaptor proteins. Reprint with permission from: Taro Kawai et al., Signaling to NF-kB by Toll-like receptors, 2007

metabolism produced by microbial pathogens [24]. Several microbial components, which are known as pathogen-associated molecular patterns (PAMPs), can induce the immune responses, and LPS plays a dominant role. Besides, Gram-positive bacteria with specific human leukocyte antigen (HLA) class II alleles, cell-wall structures such as flagellin and curli, and unmethylated CpG sequences in naked bacterial DNA may also induce the toxic shock syndromes. Expression of TLRs is not static but rather is modulated rapidly in response to pathogens, a variety of cytokines, and environmental stresses.

LPS consists of three parts: lipid A (or endotoxin), a core oligosaccharide, and an O side chain [25]. Lipid A is the main pathogen-associated molecular pattern (PAMP) of LPS. TLRs have three domains, consisting of an extracellular domain containing leucine-rich repeats, which are responsible for ligand binding, a transmembrane domain, and a cytoplasmic Toll/IL-1 receptor (TIR) domain, which is required for signal transmission [26]. As the key signaling domain, TIR domain has five adaptor molecules, namely, MyD88, MyD88-adaptor-like (MAL, also known as TIRAP), TIR-domain-containing adaptor protein-inducing IFN-b (TRIF)/TIR-domain-containing molecule 1 (TICAM1), TRIF-related adaptor molecule (TRAM, also known as TICAM2), and sterileα- and armadillo motif-containing protein (SARM) [27]. The finding of these molecules allows us to understand more comprehensive the detailed molecular description of the earliest phase of TLR signal transduction.

When the components of pathogenic microorganisms invade in the body, LPS is liberated from Gram-negative bacteria and then sensed by LPS binding protein, a soluble shuttle protein which directly binds to LPS and strongly enhances the attachment to macrophages [24, 28], and the opsonic receptor CD14, a glycosylphosphatidylinositol (GPI)-linked protein expressed on the cell surface of phagocytes [25, 29, 30]. The signal is transduced through Toll-like receptor (TLR4)-MD-2 complex [31]. MD-2 is a key molecule of LPS signaling and associates with the extracellular portion of TLR4, followed by oligomerization of TLR4 [29, 32].

After binding to its ligands, TLRs dimerize and undergo conformational changes required for the recruitment of TIR-domain-containing adaptor molecules to the TIR domain of the TLRs [15].

The differential responses mediated by distinct TLR ligands can be explained in part by the selective usage of these adaptor molecules, and the TIR domain of TLR4 is critical for signal transduction. The cytoplasmic face of TLR4 is quite unique among TLRs because it utilizes four TIR-domain-containing adaptor molecules: MyD88, Mal, TRIF, and TRAM [33]. TLR4 can signal through two distinct signaling pathways, both MYD88 (myeloid differentiation primary response gene 88)-dependent and MYD88-independent, TRIF-dependent pathways [34, 35] (Fig. 7.2). Signaling via MYD88 involves the rapid activation of NF-κB, which leads to the production of pro-inflammatory cytokines, while signaling via TRIF involves a slower activation of NF-κB and IFN regulatory factor3 (IRF3), leading to the production of type I IFN, IFN-inducible gene products, and the full innate immune response [35]. Upon LPS stimulation, MYD88 is recruited to the surface of TLR4 by a sorting adaptor, TIRAP (TIR-domain-containing adapter protein) [36], and then MYD88 recruits and activates a death domain-containing kinase, IL-1 receptor-associated kinase-4 (IRAK-4). In addition, IRAK-4 is responsible for the subsequent recruitment, activation, and degradation of IRAK-1 [37, 38]. After phosphorylated by the activated IRAK-4, IRAK-1 is subsequently associated with TNFR-associated factor 6(TRAF6) [39]. TRAF6, binding with UBC13 (ubiquitin-conjugating enzyme 13) and UEV1A (ubiquitin-conjugating enzyme E2 variant 1 isoform A) [40], actives TAK1 (transforming growth factor-β-activated kinase 1). TAK1 then activates downstream IKK (IκB kinase) and MAPK (mitogen-activated protein kinase) pathways. IKKα, IKKβ, and IKKγ form a complex and phosphorylate IκB protein, which can keep NF-κB inactive when binding to it [41]. TAK1 then phosphorylates IKKβ and MAP kinase kinase6 (MKK6), which modulates the activation of NF-κB and MAPK, resulting in induction of genes involved in inflammatory responses. Besides, activation of the MAPK pathways leads to the phosphorylation of Jun proteins and p38 and then heterodimerizes with the MAPK phosphorylation product to form activation protein 1 (AP-1), another transcription factor that affects the expression of pro-inflammatory cytokines [42, 43]. In addition to NF-κB and MAPK, IκB-ζ and IRF5 (interferon regulatory factor 5) are two important factors downstream of MyD88 [42, 43]. Upon stimulation with TLR ligands, IRF-5 translocates into the nucleus and binds potential IFN-stimulated response element (ISRE) motifs present in the promoter regions of cytokine genes [44] (Fig. 7.3).

As for the MYD88-independent pathway (also called the TLR4/TRIF-dependent pathway), it is initiated by another TIR-domain-containing adaptor, TRIF [45], which plays a key role in the activation of transcription factor IRF3, and the late-phase activation of NF-κB and MAPK [35]. TRAM (TRIF-related adaptor molecule) functions as a sorting adaptor to recruit TRIF to the plasma membrane, where TLR4 is located. Then, RAF3, a member of the TNF receptor-associated factor, recruited by TRIF, associates with TANK (TRAF family member-associated NF-κB activator), TBK1 (TANK binding kinase 1), and IKKi and thereby induces IFN-β

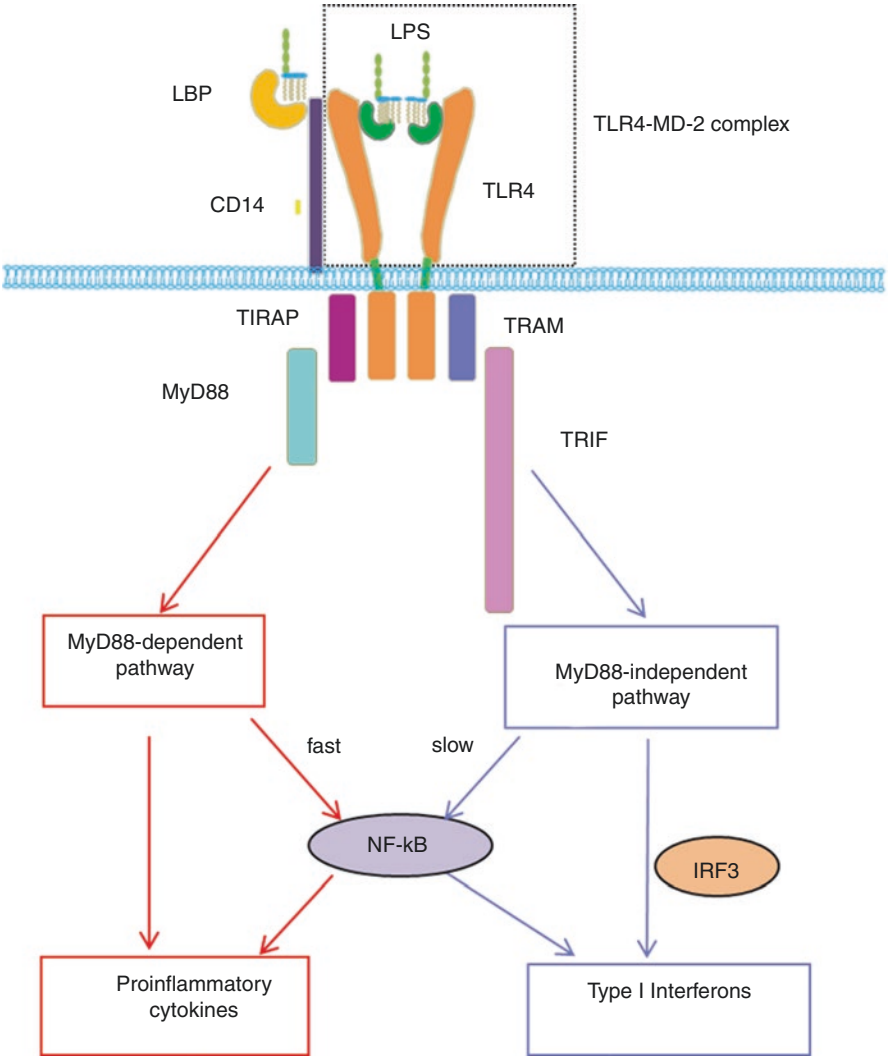

Fig. 7.2 Overview of LPS/TLR4 signaling. (Reprint with permission from Yong-Chen Lu et al., LPS/TLR4 signal transduction pathway. Cytokine, 2008)

[35, 46, 47]. Besides, the C-terminal region of TRIF mediates the interaction with RIP1 (receptor-interacting protein1) and activates the downstream signaling to produce AP-1 and NF-κB. NF-κB, together with IRF3, activates the transcription of target genes [33, 48] (Fig. 7.4).

Both the pathways result in the release of many inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, and IL-8. TNF-a and IL-1 are released during the first 30–90 min after exposure to LPS and in turn activate a second level of inflammatory cascades, as well as upregulating cell adhesion molecules

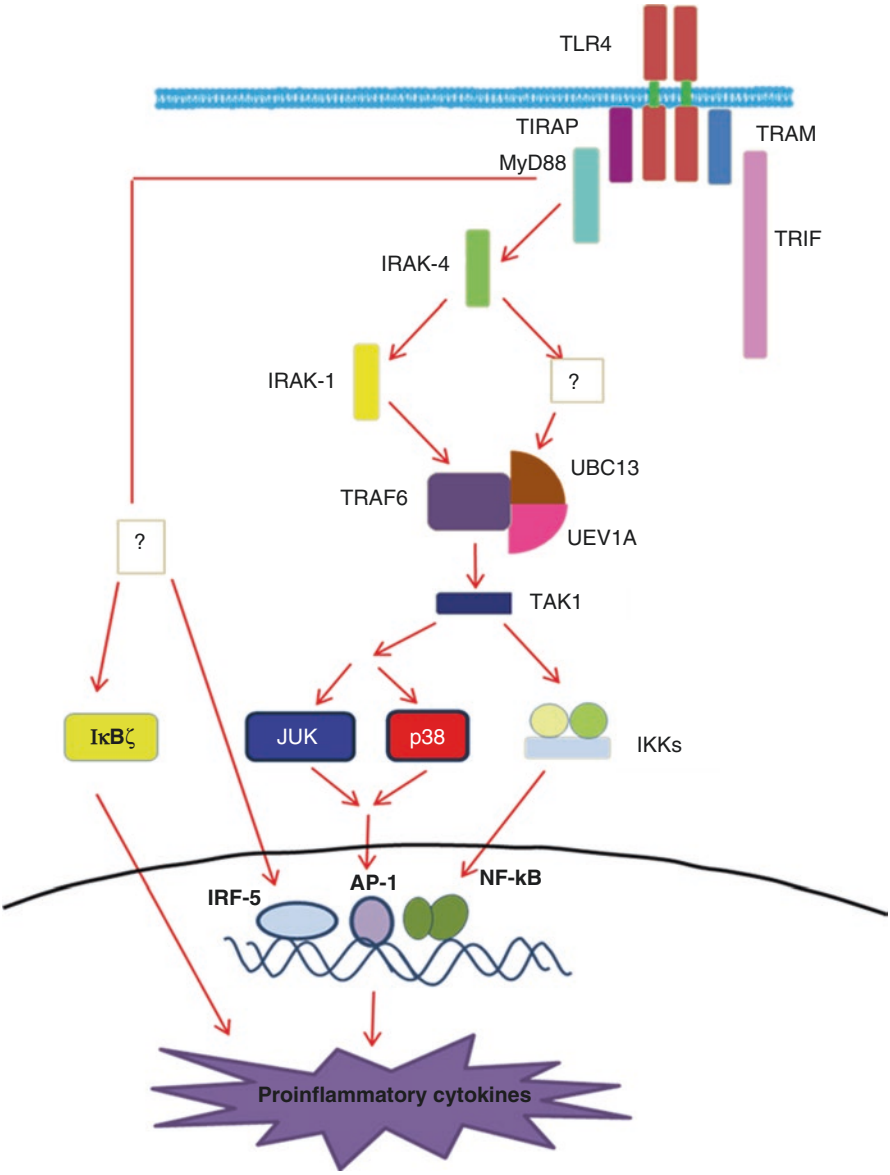

Fig. 7.3 The MyD88-dependent pathway. Yong-Chen Lu et al., Cytokine, 2008. (Reprint with permission from Yong-Chen Lu et al., LPS/TLR4 signal transduction pathway. Cytokine, 2008)

that result in the initiation of inflammatory cell migration into tissues [3]. Although the signaling pathway of TLR4 has been explicitly elaborated, the clinical treatment of sepsis remains unsatisfactory, and because too much activation of LPS/TLR4 signaling can lead to sepsis and chronic inflammatory disorders, we need to look for more receptors to help us to optimize the treatment.

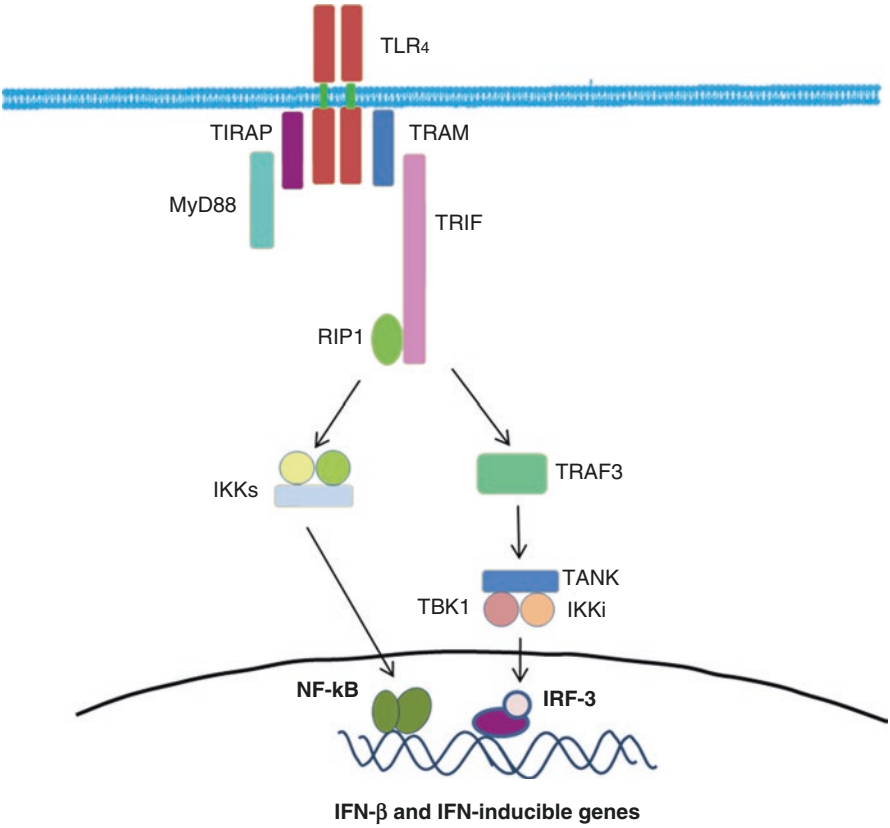

Fig. 7.4 The MyD88-independent pathway. Yong-Chen Lu et al., Cytokine, 2008. (Reprint with permission from Yong-Chen Lu et al., LPS/TLR4 signal transduction pathway. Cytokine, 2008)

7.3 The Present Mechanism Research and Treatment Progress of TLR4

7.3.1 RAGE

RAGE, the receptor for advanced glycation end products, is a central signaling molecule in the innate immune system and is involved in the onset and sustainment of the inflammatory response, which has an intimate connection with TLRs [49]. RAGE is a member of the immunoglobulin superfamily of cell surface receptors and has a single transmembrane domain and a short cytoplasmic domain which is essential for signal transduction [50]. In healthy animals, RAGE expression is very low in most cell types; on the contrary, elevated expression in RAGE and its ligands is observed in different disease states. The ligands include the advanced glycation end products (AGEs) [51], members of the S100 protein family [52], high-mobility group protein box-1 (HMGB1) [53, 54], and so on. Then, ligand engagement of

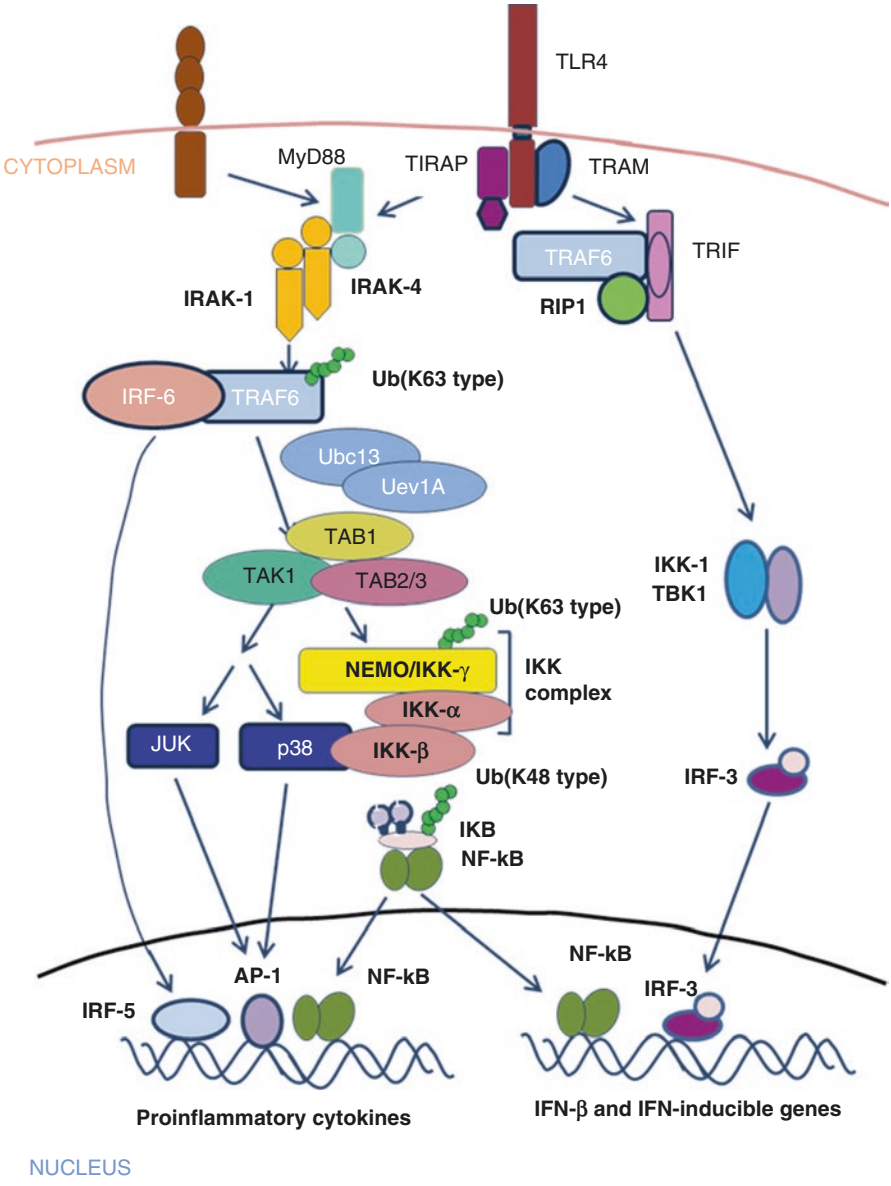

Fig. 7.5 The TLR4 signaling pathway. (Reprint with permission from Shizuo Akir et al., Pathogen recognition and innate immunity. Cell 2006)

RAGE activates multiple signaling pathways, such as environment and cell type, and covers Ras-extracellular signal-regulated kinase1/2 (ERK1/2) [55], stress-activated protein kinase/c-Jun-NH2-terminal kinase (SAPK/JUK), and p38 mitogen-activated protein kinase (MAPK) pathways [53], resulting in the activation of NF-κB [56] (Fig. 7.5).

Recently, researchers found that TLRs and RAGE share common ligands and bind preferentially to one receptor versus the other under certain physiological or pathological conditions [49].

A number of TLR ligands form complexes with HMGB1. Interestingly, these complexes elicit stronger inflammatory responses compared to HMGB1 or partner molecule alone via mechanisms that appear to involve co-activation of TLR and RAGE signaling [57]. Hreggvidsdottir et al also reported the RAGE-TLR cooperation in the inflammatory response induced by HMGB1 complexes with either the TLR4 ligand, LPS, or the TLR2 ligand, Pam3CSK4, showing that this is mediated by activation of TLR2 or TLR4, respectively [58]. According to Yamasoba et al, at-HMGB1 and ds-HMGB1 in the peripheral tissue are capable of causing mechanical hyperalgesia via activation of RAGE and TLR4 and also showed the cooperation between RAGE and TLR4 [59].

S100A8/A9 are members of the S100 protein family. S100A8/A9 heterodimeric complexes have been identified as ligands of RAGE in a co-immunoprecipitation study in isolated cardiac myocytes from mice. Boyd et al. found that RAGE co-immunoprecipitated with S100A8 and S100A9 proteins in the tissue lysates [60]. Likely, S100A8/A9 is also an endogenous ligand for TLR4. In an in vivo study, Vogl et al. demonstrated that this protein complex binds directly to TLR4 with a binding affinity of Kd 1.1–2.5 × 10^{-8}M and is important in the pathogenesis of sepsis upstream of TNF-α action [61]. Ichikawa et al. compared the S100A8/A9 interaction with TLR4 and RAGE and found that it is RAGE but not TLR4 that associates with S100A8/A9 protein in colon tumor cells [62]. Indeed, recent studies also found that RAGE and TLR4 share the common protein, S100A8/A9 protein, to activate the downstream pathways [58, 63].

As for LPS, recent studies also demonstrate that LPS can interact with RAGE. LPS binding to RAGE induced similar cellular responses as that seen with TLR4 binding both in vitro and in vivo, suggesting that soluble RAGE may be a therapeutic tool for LPS-induced sepsis and septic shock.

Ras related in brain (Rab) proteins are small guanosine triphosphatases (GTPases) belonging to the Ras superfamily that regulate vesicular formation, movement, and fusion processes [64]. Di Wang et al. showed in their study that Rab10 expression can upregulate LPS-induced activation of multiple intracellular signaling pathways and Rab10 primarily co-localizes with TLR4 in the surface of membrane, suggesting that rab10 is a positive regulator of TLR4 signaling, possibly by promoting transport of TLR4 from the Golgi to plasma membrane [64–66]. Unfortunately, the underlying mechanism and relationship between LPS, RAGE, and Rab10 remain unclear till now.

Further studies are needed to determine whether there is convergence between RAGE and TLR4, and new therapeutic targets may be found based on the relationship between RAGE and TLR4.

7.3.2 EGFR

The signaling network composed of the epidermal growth factor (EGF) family of hormones and their receptors regulates the proliferation and differentiation of many tissue types [67]. The epidermal growth factor receptor (EGFR, also known as

HER1 or ErbB1) belongs to the ErbB family of cell surface receptor tyrosine kinases (RTKs) and is a key player in the regulation of cell proliferation, differentiation, survival, and migration. Overexpression and mutational changes of EGFR have been identified in a variety of pro-inflammatory reactions, and the regulation of EGFR signaling plays a critical role in pro-inflammatory cytokine development and progression [68].

Upon activation, EGFR triggers homodimerization or heterodimerization of this receptor with other ErbB members and activates downstream effectors. One signal transduction pathway of EGFR activation is the RAS-RAF-MEK-ERK-MAPK signaling cascade, and the other is the PI3K-AKT-mTOR signaling pathway, leading to cell proliferation [68–71] (Figs. 7.6 and 7.7).

Recently, researchers payed more attention to the inhibition of EGFR signaling pathway to decrease the induction of TNF-α to alleviate the pro-inflammatory response cascade. Küper et al. found that Toll-like receptor 4 activates NF-κB and MAP kinase pathways to regulate expression of pro-inflammatory COX-2 in renal medullary collection duct cells [72]. De Sarmishtha et al. found that there is a cross signaling between EGFR and TLR4. NF-κB activation in response to EGF requires EGFR, TLR4, and two downstream proteins [34]. Conversely, EGFR activation is required for LPS-induced NF-κB activation by inhibition of EGFR kinase activity using the EGFR inhibitor erlotinib in HME cells. It is momentous that erlotinib, an EGFR inhibitor, used extensively in cancer treatment, is also beneficial in depressing the inflammatory signals triggered by LPS. Interestingly, according to Chattopadhyay et al., EGFR is required for TLR4 signaling by the TRIF branch of TLR4 signaling instead of the NF-κB pathway in myeloid cells [29]. Moreover, EGFR inhibitor, gefitinib, inhibited both the basal and the enhanced levels of IL-1βsecretion. Meanwhile, Sun et al. also highlight the role of EGFR inhibitor in LPS-induced endotoxemia [69]. In this study, they demonstrated that activation of EGFR is the key

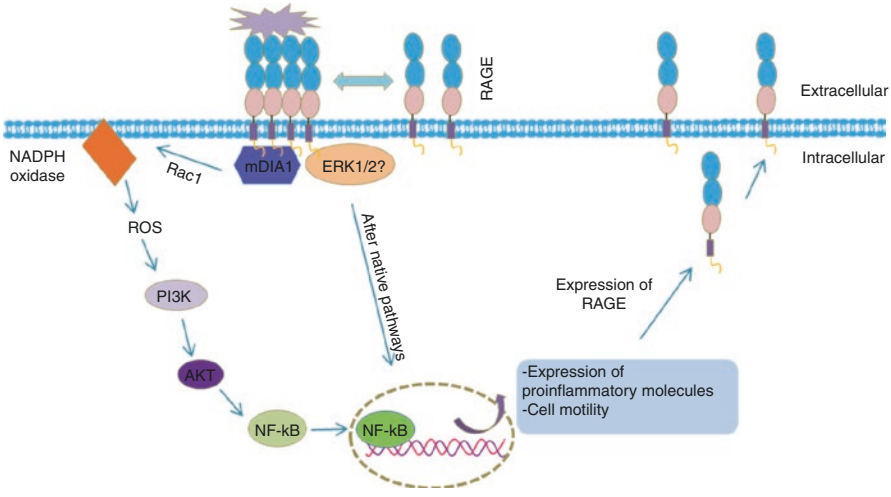

Fig. 7.6 Preassembly and activation of RAGE. Reprint with permission from: Gunter Fritz et al., RAGE: a single receptor fits multiple ligands. Trends in Biochemical Science, 2011

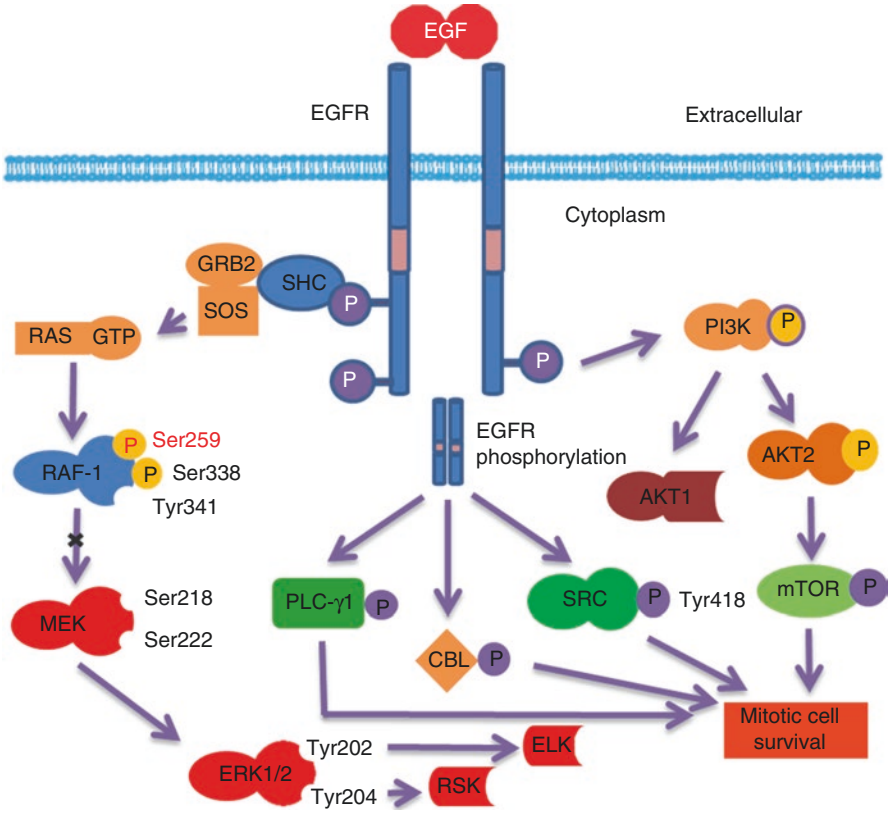

Fig. 7.7 Schematic illustration of EGFR signaling pathways during mitosis and interphase. (Reprint with permission from: Ping Wee et al., EGF stimulates the activation of EGF receptors and the selective action of major signaling pathway during mitosis. Cellular Signaling, 2015)

step for the production of TNF-α induced by LPS via promoting p38 and ERK1/2 phosphorylation. While EGFR is regularly expressed in cardiomyocytes and cardiac-derived TNF-α is involved in promoting cardiovascular failure in sepsis, erlotinib may have a protective effect in LPS-induced myocardial dysfunction.

Besides the inhibitor of EGFR such as erlotinib and gefitinib or so, gene transfer therapy is also a potential effective way for the treatment of sepsis. Liu et al. elaborated a novel EGFR-dependent mechanism for regulating TLR, that is, EGFR-Erk signaling, and show that targeted disruption of EGFR signaling ameliorates the pDNA-mediated inflammatory [73].

7.3.3 HMGB1

High-mobility group box 1(HMGB1), a nonhistone chromosomal protein, is an evolutionarily conserved protein that is abundantly distributed and exists in nuclear, cytoplasmic, and membrane-bound forms in most eukaryotic cells [74–76]. When

sterile inflammation and invasive threat occurs, HMGB1 would release into the extracellular milieu [77]. Activated immunocompetent cells like monocytes actively secrete HMGB1 after exposure to pathogen- or damage-associated molecular patterns including LPS, TNF-α, or IL-1. Its detection in serum is delayed by several hours compared with other pro-inflammatory cytokines such as IL-1 and TNF-α.

It is reported by Wang et al. that the serum concentrations of HMGB1 will rise after a delay of about 24–48 h when mice were injected with LPS, long after the initial peak of IL-1 and TNF-α had declined [78]. Mice could be rescued from LPS-induced shock by administering an antibody to HMGB1, even when this was provided up to 2 h after the lethal injection, suggesting that serum HMGB1 levels may be a convenient marker of disease severity and clinical intervention by blocking or neutralizing HMGB1 might be a viable option [76, 78]. Yang et al. gave the same conclusion as well. Besides, he also found that acute shock and tissue injury are mediated by TNF, while lethal organ failure and epithelial barrier failure without shock are mediated by HMGB1 [79]. According to Levy et al., neutralizing antibodies to HMGB1 are profoundly protective in their injury model, and systemic levels of HMGB1 are elevated in both TLR4-WT and TLR4-Mu animals as early as 1 h after injury [80]. These findings may represent a more tractable target for intervention in patients with sepsis.

Hwang et al. [74] found that SIRT1, a human homolog of the *Saccharomyces cerevisiae* protein silent information regulator 2, directly interacted with HMGB1 and thereby inhibited HMGB1 release to improve survival in an experimental model of sepsis via its N-terminal lysine residues. Together with the present finding that pharmacological activation of SIRT1 by resveratrol significantly inhibits HMGB1 release and reduces septic liver injury [81], targeting of SIRT1 in inflammation-related diseases may elicit therapeutic effects in sepsis by decreasing the extra cellular level of HMGB1. The recombinant HMGB1 A box also acts as one kind of possible treatments. Yang et al. reported that the formation of the HMGB1 A box by proteolytic degradation at the site of inflammation could provide a regulatory influence to protect against HMGB1-mediated toxicity [79].

Taken together, HMGB1 may occupy a major pathogenic role on the final common pathway to death, specific inhibition of septic HMGB1 therapeutically reverses lethality of established sepsis, and HMGB1 inhibitors can be administered in a clinically relevant time frame.

7.3.4 MIF

MIF, the macrophage migration inhibitory factor, also has been identified as an attractive therapeutic target in sepsis. According to Calandra et al., MIF is a critical component of the immune system and plays an important part in the control of inflammatory response. Experiments performed in TNF-α knockout mice revealed the part played by MIF in sepsis in the absence of this pivotal cytokine of inflammation. Anti-MIF antibody protected TNF-α knockout from lethal peritonitis induced by cecal ligation and puncture (CLP) and protected normal mice from lethal peritonitis induced by both CLP and *Escherichia coli*, suggesting that anti-MIF strategies

might someday find utility in the management of sepsis [82]. MIF also seems to mediate the pathophysiology changes caused by Gram-positive bacteria [83], leading a more broad application in MIF. Besides, MIF has an interesting relationship with glucocorticoids, which are normally thought of as being anti-inflammatory and widely used in clinical sepsis, as low doses of glucocorticoids paradoxically induce macrophage MIF. Once released, MIF acts to override glucocorticoid-mediated inhibition of cytokine secretion by LPS-stimulated monocytes and to overcome glucocorticoid protection against lethal endotoxemia [84]. Bozza et al. reported that mice with a targeted disruption of the MIF gene are resistant to endotoxin-induced lethal shock. All these findings suggest that the counteraction or neutralization of MIF may serve as an adjunct therapy in sepsis [85].

7.4 Conclusion and Expectation

Sepsis, severe sepsis, and septic shock, as one of the oldest and most pressing problems in intense care unit, need new therapies to increase the survival rate. With advances in intensive care, increased awareness, and proposition of new evidence-based guidelines, clinicians have taken enormous efforts in reducing the risk of imminent death associated with sepsis. However, the results are unsatisfactory. There still more and more people suffer from severe sepsis and septic shock, especially in children and elderly. The major unanswered question is about the onset and persistence of cellular dysfunction and organ failure, which are the ultimate causes of death. As it is increasingly difficult to carry out studies on the patients with sepsis, developing a better understanding of its pathophysiology and finding more possible therapeutic targets are clearly in order. Only with early diagnosis and expedited treatment based on evidence-based medicine can sepsis morbidity and mortality be decreased. There is still a long way we have to explore.

References

1. Vincent J, et al. Sepsis definitions: time for change. Lancet. 2013;381(9868):774–5.
2. Mukherjee V, Evans L. Implementation of the surviving sepsis campaign guidelines. Curr Opin Crit Care. 2017;23(5):412–6.
3. Cohen J. The immunopathogenesis of sepsis. Nature. 2002;420(6917):885–91.
4. Singer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA. 2016;315(8):801.
5. Manu Shankar-Hari DAH. Differences in impact of definitional elements on mortality precludes international comparisons of sepsis epidemiology-a cohort study illustrating the need for standardized reporting. Crit Care Med. 2016;44(12):2223–30.
6. Allan J, Walkey TL, Lindenauer PK. Trends in sepsis and infection sources in the United States. Ann Am Thorac Soc. 2015;12(2):216–20.
7. Yuki K, Murakami N. Sepsis pathophysiology and anesthetic consideration. Cardiovasc Hematol Disord Drug Targets. 2015;15(1):57–69.
8. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013;369(9):840–51.
9. Linde-Zwirble WT, Angus DC. Severe sepsis epidemiology: sampling, selection, and society. Crit Care. 2004;8(4):222–6.

10. Weber GF, Swirski FK. Immunopathogenesis of abdominal sepsis. Langenbeck Arch Surg. 2014;399(1):1–9.
11. Schorr CA, Zanotti S, Dellinger RP. Severe sepsis and septic shock. Virulence. 2013;5(1):190–9.
12. Rhodes A, et al. Surviving sepsis campaign. Crit Care Med. 2017;45(3):486–552.
13. Opal SM, Girard TD, Ely EW. The immunopathogenesis of sepsis in elderly patients. CID. 2005;41(7):504–12.
14. Henry J, Jacobsen W, Watkins LR, Hutchinson MR. Discovery of a novel site of opioid action at the innate immune pattern-recognition receptor TLR4 and its role in addiction. Int Rev Neurobiol. 2014;118:129–63.
15. Shizuo Akira SU, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.
16. Anderson KV, Bokla L, Nusslein-Volhard C. Establishment of dorsal-ventral polarity in the drosophila embryo: the induction of polarity by the toll gene product. Cell. 1985;42:791–8.
17. Jiménez-Dalmaroni MJ, Gerswhin ME, Adamopoulos IE. The critical role of toll-like receptors — from microbial recognition to autoimmunity: a comprehensive review. Autoimmun Rev. 2016;15(1):1–8.
18. Kawai T, Akira S. TLR signaling. Cell Death Differ. 2017;13:816–25.
19. Takeuchi O, Hoshino K, Kawai T. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999;11: 443–51.
20. Hayashi F, Smith KD, Ozinsky A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001;410:1099–103.
21. Heil F. Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. Science. 2004;303(5663):1526–9.
22. Sandra S, Diebold TKHH. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303:1529–31.
23. Yarovinsky F, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science. 2005;308(5728):1626–9.
24. Janeway CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20(1):197–216.
25. Raetz CRH, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem. 2002;71(1):635–700.
26. Kawai T, Akira S. Signaling to NF-κB by Toll-like receptors. Trends Mol Med. 2007;13(11):460–9.
27. O'Neill LAJ, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol. 2007;7(5):353–64.
28. Wright SD, Tobias PS, Ulevitch RJ. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. J Exp Med. 1989;170:1231–41.
29. Chattopadhyay S, et al. EGFR kinase activity is required for TLR4 signaling and the septic shock response. EMBO Rep. 2015;16:1535–47.
30. Schumann RR, Leong SR, Flaggs GW. Structure and function of lipopolysaccharide binding protein. Science. 1990;249(4975):1429–31.
31. Nagai Y, et al. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. Nat Immunol. 2002;3(7):667–72.
32. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol. 1995;13:437–57.
33. Rowe DC, et al. The myristoylation of TRIF-related adaptor molecule is essential for Toll-like receptor 4 signal transduction. PNAS. 2006;103(16):6299–304.
34. De S, et al. Erlotinib protects against LPS-induced endotoxicity because TLR4 needs EGFR to signal. Proc Natl Acad Sci. 2015;112(31):9680–5.
35. Lu Y, Yeh W, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine. 2008;42(2):145–51.
36. Horng T, Barton GM, Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol. 2001;2(9):835–41.

37. Suzuki N, Suzuki S, Duncan GS. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature. 2002;416:750–4.
38. Lye E, et al. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. J Biol Chem. 2004;279(39):40653–8.
39. Li S, et al. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc Natl Acad Sci U S A. 2002;99(8):5567–72.
40. Lomaga MA, et al. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev. 1999;13(8):1015–24.
41. Janssens S, Beyaert R. A universal role for MyD88 in TLR/IL-1R-mediated signaling. Trends Biochem Sci. 2002;27(9):474–82.
42. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001;410:37–40.
43. Treisman R. Regulation of transcription by MAP kinase cascades. Curr Opin Cell Biol. 1996;8:205–15.
44. Takaoka A, Yanai H, Kondo S. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature. 2005;434:243–9.
45. Hoebe K, et al. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature. 2003;424:743–8.
46. Takeda K, Akira S. TLR signaling pathways. Semin Immunol. 2004;16(1):3–9.
47. Oganesyan G, et al. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. Nature. 2005;439(7073):208–11.
48. Kagan JC, et al. TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-β. Nat Immunol. 2008;9(4):361–8.
49. Ibrahim ZA, et al. RAGE and TLRs: relatives, friends or neighbours? Mol Immunol. 2013;56(4):739–44.
50. Fritz G. RAGE: a single receptor fits multiple ligands. Trends Biochem Sci. 2011;36(12):625–32.
51. Hori O, Brett J, Slattery T. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. J Biol Chem. 1995;270(43):25752–61.
52. Leclerc E, et al. Binding of S100 proteins to RAGE: an update. Biochim Biophys Acta. 2009;1793(6):993–1007.
53. Taguchi A, Blood DC, Toro GD. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature. 2000;405:354–60.
54. Bierhaus A, Nawroth PP. Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications. Diabetologia. 2009;52(11):2251–63.
55. Huttunen HJ, Kuja-Panula J, Rauvala H. Receptor for advanced glycation end products (RAGE) signaling induces CREB-dependent chromogranin expression during neuronal differentiation. J Biol Chem. 2002;277(41):38635–46.
56. Du Yan S, Schmidt AM, Anderson GM. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors binding proteins. J Biol Chem. 1993;269(13):9889–97.
57. Hreggvidsdottir HS, Lundberg AM, Aveberger A. High mobility group box protein 1 (HMGB1)-partner molecule complexes enhance cytokine production by signaling through the partner molecule receptor. Mol Med. 2012;18:224–30.
58. Hreggvidsdottir HS, Ostberg TH. The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. J Leukoc Biol. 2009;86:655–62.
59. Yamasoba D, et al. Peripheral HMGB1-induced hyperalgesia in mice: redox state-dependent distinct roles of RAGE and TLR4. J Pharmacol Sci. 2016;130(2):139–42.
60. Boyd JH, et al. S100A8 and S100A9 mediate endotoxin-induced cardiomyocyte dysfunction via the receptor for advanced glycation end products. Circ Res. 2008;102(10):1239–46.
61. Vogl T, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med. 2007;13(9):1042–9.
62. Ichikawa M, et al. S100A8/A9 activate key genes and pathways in colon tumor progression. Mol Cancer Res. 2011;9(2):133–48.

63. van Lent PLEM, et al. Myeloid-related proteins S100A8/S100A9 regulate joint inflammation and cartilage destruction during antigen-induced arthritis. Ann Rheum Dis. 2008;67(12):1750–8.
64. English AR, Voeltz GK. Rab10 GTPase regulates ER dynamics and morphology. Nat Cell Biol. 2012;15(2):169–78.
65. Wang D, et al. Ras-related protein Rab10 facilitates TLR4 signaling by promoting replenishment of TLR4 onto the plasma membrane. Proc Natl Acad Sci. 2010;107(31):13806–11.
66. Zou W, et al. RAB-10-dependent membrane transport is required for dendrite arborization. PLoS Genet. 2015;11(9):e1005484.
67. David II, Stern DF. Specificity within the EGF family/ErbB receptor family signaling network. BioEssays. 1998;20:41–8.
68. Morandell S, et al. Quantitative proteomics and phosphoproteomics reveal novel insights into complexity and dynamics of the EGFR signaling network. Proteomics. 2008;8(21):4383–401.
69. Sun X, et al. The activation of EGFR promotes myocardial tumor necrosis factor-α production and cardiac failure in endotoxemia. Oncotarget. 2015;6(34):35478–95.
70. Wee P, et al. EGF stimulates the activation of EGF receptors and the selective activation of major signaling pathways during mitosis. Cell Signal. 2015;27(3):638–51.
71. Hackel PO, et al. Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr Opin Cell Biol. 1999;11(2):184–9.
72. Kuper C, Beck FX, Neuhofer W. Toll-like receptor 4 activates NF-B and MAP kinase pathways to regulate expression of proinflammatory COX-2 in renal medullary collecting duct cells. Am J Physiol Renal Physiol. 2011;302(1):F38–46.
73. Liu K, Anderson GP, Bozinovski S. DNA vector augments inflammation in epithelial cells via EGFR-dependent regulation of TLR4 and TLR2. Am J Respir Cell Mol Biol. 2008;39(3):305–11.
74. Hwang JS, et al. Deacetylation-mediated interaction of SIRT1-HMGB1 improves survival in a mouse model of endotoxemia. Sci Rep. 2015;5(1):15971.
75. Muller S, Ronfani L, Bianchi ME. Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function. J Intern Med. 2004;255(3):332–43.
76. Gardella S, et al. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. EMBO Rep. 2002;3(10):995–1001.
77. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. Annu Rev Immunol. 2011;29(1):139–62.
78. Wang H, Bloom O, Zhang M. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285(5425):248–51.
79. Yang H, et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. PNAS. 2004;101(1):296–301.
80. Levy RM, et al. Systemic inflammation and remote organ injury following trauma require HMGB1. Am J Physiol Regul Integr Comp Physiol. 2007;293(4):R1538–44.
81. Andreas Rickenbacher JHJP. Fasting protects liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. J Hepatol. 2014;61:301–8.
82. Calandra T, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med. 2000;6(2):164–70.
83. Calandra T, et al. Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of gram-positive bacteria. Proc Natl Acad Sci U S A. 1998;95(19):11383–8.
84. Calandra T, Bernhagen J, Christine M. MIF as a glucocorticoid-induced modulator of cytokine production. Nature. 1995;377(7):68–71.
85. Bozza M, Satoskar AR, Lin G. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. J Exp Med. 1999;189(2):341–6.

Pro-resolution of Inflammation: New Hints to Manage Sepsis?

8

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Abstract

Sepsis is newly defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. The pathophysiological mechanism of sepsis is highly complex, and the mortality of in-patients suffering from sepsis is more than 10%. Severe unmanaged inflammation and inappropriate immune response characterize sepsis. Anti-inflammation therapies alone are not successful for the reason that disbalance of anti-inflammatory and pro-resolving agents. In the recent researches, the host responses during the course of self-resolving infections are found to have the involvements of specialized pro-resolution mediators (SPMs), namely, lipoxins, resolvins, protectins and maresins. These endogenous lipid metabolites are core signal molecules in the resolution of inflammation, playing a key role in regulating the inflammation and promoting return to homeostasis. Besides, heme oxygenase-1 (HO-1, a sensitive marker for oxidative stress) is also known for upregulation in inflammation profiling. Carbon monoxide, synthesized by HO-1, performs multiple stances of anti-inflammation and pro-resolution along with the SPMs. If the potentially beneficial effects of these mediators would be well evaluated in clinical trials, they present encouraging new hints in managing infectious maladies especially sepsis.

Keywords

Sepsis · Specialized pro-resolution mediators · Pro-resolution

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8.1 Introduction

Sepsis is a complicated series of pathophysiological and biochemical disbalance due to the uncontrolled inflammatory response to the invasive pathogens. Its newest definition is defined as organ dysfunction due to infection, which remains a major public health concern that is associated with poor clinical outcomes (in-hospital mortality is more than 10%) and substantial healthcare expenditures [1]. Data showed that the cost of medical care for sepsis patients was more than $20 billion (5.2%) of total US hospital costs in 2011 [2]. Although the accurate incidence of sepsis is unknown, the reported data are increasing [3, 4], and conservative estimates indicate that sepsis is a leading cause of mortality in the US hospitals and critical illness in the world [5, 6].

The inflammatory host response was long considered as a passive process, which is terminated by the clearance of pro-inflammatory mediators. Recently, it is recognized that the initiation and cessation of immune and inflammatory responses is an initiative and symphony process. The pathogenesis of sepsis is intricate. The early stages of sepsis are characterized by excessive generation of inflammatory mediators; however, as sepsis develops into severe chronic sepsis, immunosuppression dominates. This demonstrates that excessive uncontrolled inflammation and inappropriate immune response characterize sepsis [7]. The new lights into sepsis contribute to the explanation of failure in current treatment strategies, including inhibition of the activation phase of the acute inflammatory response to infection (e.g. glucocorticoids, nonsteroidal anti-inflammatory drugs and anti-TNF-α drugs) [8]. Therefore, it's urgent and beneficial to explore new paradigms in managing sepsis.

As of recent studies relating the host response to a self-resolving infection, it is brought into light that a genus of endogenous bioactive lipid mediators (SPMs) is produced by the innate immune system cells. A process of stereoselective enzyme conversion of essential fatty acids including arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and n-3 docosapentaenoic acid (n-3 DPA) produces these molecules. The synthetases include cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P-450 monooxygenase. The SPMs can further be subdivided into lipoxins, resolvins, protectins, maresins and immunoresolvents (RvTs, PCTRs and MCTRs) (Fig. 8.1) [9–12].

Through the G-protein-coupled receptor-dependent manner, these SPMs exhibit the fundamental bioactivities in the maintaining of host responses. Hence, the productions of pro-inflammatory cytokines (TNF-α and IL-1β) and chemokines are inhibited, and the downregulation of inflammation-initiating eicosanoids (prostaglandins and leukotrienes) is promoted. This as well regulates the leukocyte infiltration and stimulates the macrophage efferocytosis of apoptotic cells and debris [10, 11]. Recent studies have shown that the SPMs exhibit beneficial impacts at the site and in course of sepsis while maintaining the homeostasis between the abundance of microorganisms and the host. In this chapter, we aim at:

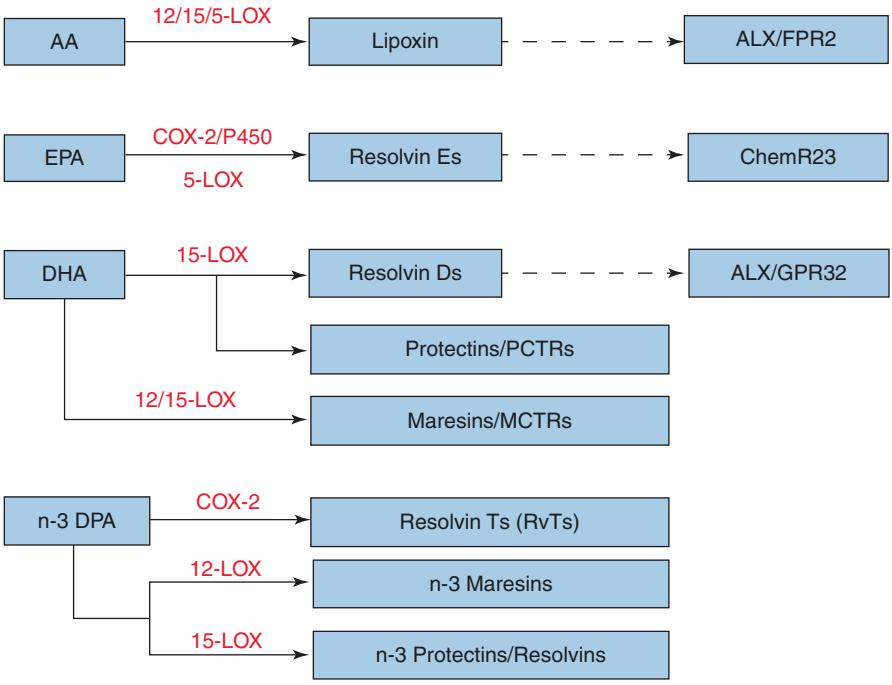

Fig. 8.1 SPMs derived from essential fatty acids and their receptors

(1) The functions of SPMs in sepsis.
(2) Because aspirin partakes in the synthesis of lipoxins and resolvins, studies related to aspirin-triggered lipid mediators are included [13].
(3) Protective effects of carbon monoxide (CO) on inflammation and its impacts of enhanced productions of pro-resolving lipids [14, 15]. Therefore carbon monoxide and its synthetase HO-1 are summarized.
(4) Since Annexin (A1) is a stress response protein, the current evidence for beneficial pro-resolving effects of this mediator in sepsis is also addressed in this chapter [16].

8.2 Mediators Related to Resolution of Inflammation

8.2.1 Lipoxins

Lipoxins (trihydroxy-tetraene-containing eicosanoids) are derived from the omega-6 polyunsaturated fatty acid arachidonic acid (AA) through sequential reactions involving lipoxygenase enzymes, including 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX) and 15-lipoxygenase type 1 (15-LOX-1). There are two series of lipoxins, lipoxin-A4 (LXA4) and lipoxin-B4 (LXB4) [17]. We mainly summarize the activities of LXA4 in this chapter.

Lipoxins exert effects through a G-protein-coupled receptor, formyl peptide receptor-2 (FPR2/ALX). This receptor mainly expresses on the membrane of cells in the immune system along with the resident fibroblasts and epithelial cells [18]. In general, lipoxins are potent inhibitors of inflammatory activity, inducing the resolution of leukocyte activity. They block the response to leukotrienes and chemotactic factors such as leukotriene B4 (LTB4) and platelet-activating factor [19]. These effects inhibit the infiltration of pro-inflammatory cells. The resolution of inflammation involves reduction of neutrophil recruitment, promotion of macrophage migration and augmentation of apoptotic cells efferocytosis. Lipoxins exert immune-modulatory activity as well [17, 18].

LXA4, treated after injury, was proved to limit inhaled LPS-induced lung injury [20]. In other experimental models, 5 h after caecal ligation and puncture (CLP), LXA4 was administered to rats which resulted in decreasing of plasma IL-6, chemokine (C-C motif) ligand 2 (CCL2) and IL-10 and reducing NF-κB activity in peritoneal macrophages. LXA4 further enhanced the phagocytosis of macrophage recruited to the peritoneum and reduced bacterial load in the blood. Hence, LXA4 improved the mortality of this CLP model [21].

Further study showed that flavocoxid, a dual inhibitor of COX-2 and 5-LOX, reduces the expression of NF-κB, COX-2 and 5-LOX which improves survival rate in a murine CLP sepsis model. Plasma IL-10 and LXA4 concentrations are increased, while tumour necrosis factor-α (TNF-α), IL-6, LTB4 and PGE2 are decreased [22].

Neutrophil phagocytosis involving the Fc receptor I (CD64) is increased and enhanced by LXA4. Meanwhile, LXA4 decreases the release of the exotoxin pyocyanin by *Pseudomonas aeruginosa*, reducing its pathogenicity [23]. Moreover, LXA4 blocks trafficking of neutrophils, inhibits their adhesion and their release of azurophilic granules. And in activated T-cells, LXA4 lowers TNF release [24]. The finding shows that LXA4 not only modulates the host response but also affects bacterial toxicity [23].

A test was run to investigate the role of receptor FPR2/ALX in mediating the protective effects in sepsis using the receptor agonist BML-111. It ameliorates intestinal inflammation in septic rats. The anti-inflammatory cytokine transforming growth factor-β (TGF-β) which is supposed to protect intestinal cells from apoptotic cell death was induced [25]. In a murine model of non-lethal polymicrobial sepsis, FPR2/ALX-deficient animals developed more serious disease and exhibit higher cytokine levels and reduced recruitment of monocytes in peritoneal lavages. Treatment with an FPR2/ALX agonist protected wild type but not the knock-out mice from cardiac dysfunction [26]. These findings prove that LXA4 protect from organ dysfunction, a major cause of mortality in sepsis [27].

As known to all, acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are major common complications of severe sepsis [27]. With the inoculation of *Klebsiella pneumoniae* in mice, pulmonary sepsis occurred and induced LXA4 and FPR2/ALX expression in the lung. Later, the treatment of receptor antagonists and inhibition of 5-lipoxygenase and 15-lipoxygenase in early sepsis (1 h postinfection) even increased leukocyte migration to the infected tissues, and

survival rate increased. On the contrary, receptor agonist and LXA4 application consequently worsened early infection and reduced migration of leukocytes. But, 24 h postinfection, LXA4 improved animal survival. Here, this research demonstrates the dual role of LXA4 and highlights the time dependence when targeting the LXA4 pathway in pulmonary infection [28].

In the case of septic patients, all the mediators identified in preclinical studies and tested for the treatment in clinical trials have failed [29]. Pro-resolving lipid analysis in critically ill patients may reveal a novel orientation for treatment and bring in further insights into the pathways playing a role in the pathophysiology of sepsis. While comparing to 27 non-survival septic patients for 28 days of admission to the intensive care unit, LXA4 was significantly reduced in 39 patients that survived, but levels of this lipid were not associated with death [30].

8.2.2 Resolvins

Resolvins are also derived from omega-3 polyunsaturated fatty acids and exist as two series (D and E). E-series resolvins (RvE1 and RvE2) are products of eicosapentaenoic acid (EPA) involving 5-LOX, cytochrome P450 and aspirin-acetylated COX-2 as well. D-series resolvins (RvD1–RvD6) are synthesized from docosahexaenoic acid (DHA) metabolism involving enzymes 5-LOX/15-LOX [13, 31]. The biological activities of resolvins are similar to lipoxins. RvE1 and its analogues are more potent than LXA4 on a molar basis. RvE1 binds to the leukotriene receptor BLT1 and blocks TNF-α-stimulated NF-κB activation at the ChemR23 receptor [32].

In a mouse model of aspiration pneumonia and subsequent involvement of one lobe with *E. coli* infection, the function of RvE1 in acute lung injury was analysed and found that the RvE1, when injected before the acid injury, reduced pulmonary neutrophil infiltration and enhanced bacterial clearance. This was accompanied by lower levels of inflammatory cytokines and chemokines and marginally improved survival rate [33].

In other two murine models of acute lung injury, RvE1 enhanced cell death of neutrophils arising from the phagocytosis of opsonized *E. coli* or yeast and is mediated by the leukotriene B4 receptor BLT1. Consequently, RvE1 enhanced the resolution of the established pulmonary inflammation [34]. LXA4, RvE1 and protectin D1 increase levels of the C-C chemokine receptor 5 (CCR5) on apoptotic polymorphonuclear cells (PMNs) and thereby terminate chemokine signalling [35]. RvE1 and 15-epi-lipoxin protect macrophages from oxidative stress-associated apoptotic cell death, and this contributes to the removal of cytotoxic debris and the inflammation resolution [36, 37].

D-galactosamine-sensitized mouse endotoxin shock mode was also tested for the effects of RvD1, which counteracted the induction of high-mobility group box-1 (HMGB1) and pro-inflammatory cytokines. Hepatocyte apoptosis was suppressed, and also neutrophil immigration to the peritoneum was reduced by the effects of RvD1 [38].

In a mouse model of intraperitoneal *E. coli* peritonitis, RvD5 enhanced phagocytosis of bacteria compared to the control group. RvD1 had a similar but smaller effect. Both RvD1 and RvD5 reduced significantly titre of viable bacteria in peritoneal exudates and blood and lowered degree of hypothermia as well. Plasma levels of pro-inflammatory cytokines (TNF-α and IL-1β) were reduced by RvD1 and RvD5. Interestingly, it was found that RvD1 enhanced the antimicrobial effect of ciprofloxacin in resolving *E. coli* peritonitis and increased survival rate in this model [39].

Administration of resolvin D2 could improve outcomes of burn-related sepsis by regulating PMN chemotaxis. In a rat model of burn-related sepsis, RvD2 restored the chemotaxis of PMN to almost normal level. Furthermore, when burned rats received intravenous LPS 9 days after their burn injury, with intravenous pretreated RvD2, the survival of rats improved significantly. Similarly, RvD2 pretreatment increased survival, following caecal ligation after burn injury [40].

RvD1 injected after CLP model of sepsis increased bacterial clearance and mice survival. The numbers of peritoneal neutrophils were decreased, while CD3 T-lymphocytes apoptosis in thymus got significant improvement [41]. When AT-RvD1 was administered 1 h after the toxin in a LPS-induced acute kidney injury mouse model, renal function was improved. Lower expression of adhesion molecules, less activation of NF-κB and reduced infiltration of neutrophils have been reported as well [42].

Previous studies have shown that IV administration of RvD2 on a CLP sepsis model exhibits the following protective pro-resolution effects and increases survival rate: (1) reduce viable aerobic bacterial load in peritoneal exudates and blood; (2) reduce PMN migration into the peritoneum; (3) reduce plasma levels of IL-10 and IL-17; (4) reduce pro-inflammatory cytokine (IL-6, IL-1β, IL-23 and TNF-α) levels in plasma and peritoneum; (5) reduce concentrations of the pro-inflammatory lipids PGE2 and LTB4; while (6) increase clearance of bacteria by phagocytes in inguinal lymph nodes and in vitro; (7) enhance phagocytosis of *E. coli* by human PMN and also increase intracellular production of reactive oxygen species; and (8) increase survival as a result [43].

Inflammatory pain is mainly caused due to the activation of transient receptor potential subtype vanilloid 1 (TRPV1) and TRP ankyryn 1 (TRPA1). RvD2 is a potent inhibitor of both channels in primary sensory neurons, while RvE1 inhibits TRPV1 and RvD1 inhibits TRPA1, respectively. Hence, these lipids contribute to pain-relieving activities [44].

The potential therapeutic uses of exogenous resolvins are currently under investigation. In a recent phase 2 clinical trial involving patients with dry eye syndrome, an RvE1 analogue significantly improved signs and symptoms of corneal inflammation. This is the first trial to show the clinical efficacy of the novel class of resolvins therapeutics that stimulate resolution rather than inhibit inflammatory mediators [14].

8.2.3 Protectins

Protectins are also omega-3 polyunsaturated fatty acid derivatives, generated from docosahexaenoic acid (DHA) through 12-LOX/15-LOX-mediated pathways. Neuroprotectin D1 (NPD1) and protectin D1 (PD1) are included [45].

In a severe human influenza model, H1N1-A virus was introduced through the intratracheal route in mice, and PD1 levels were found to be reduced, suggesting that endogenous production was suppressed by the virus. When other influenza A virus strains were tested, the reduction of PD1 was found to inversely correlate with the virulence of the virus strain used. In the same model of severe human influenza, intravenous administration of PD1 reduced in vitro replication of the H1N1 influenza A virus and increased survival. Besides, When PD1 was administered in combination with the antiviral agent peramivir, a dramatic increase in survival was found compared to use of peramivir alone [46].

Protectin DX (PDX, an isomer of protectin D1) also exhibits protective effects of anti-inflammation and pro-resolution. In a CLP sepsis mice model, PDX increased overall survival rate and attenuated multiple organ injury. In addition, PDX reduced pro-inflammatory cytokines and bacterial load 24 h after CLP. Moreover, PDX promoted phagocytosis of peritoneal macrophages and increased the percentage of M2 macrophages in peritoneum of septic mice [47].

In a recent report encompassing 22 sepsis patients, it was shown that plasma levels of inflammation-initiating mediators including PGF2α and LTB4 and pro-resolving mediators, including RvE1, RvD5 and 17R-PD1, were significantly higher in non-survivors than in surviving sepsis subjects. Further analysis revealed increased respiratory failure in non-survivors. These results indicate that peripheral blood lipid mediator profiles (RvE1, RvD5 and 17R-PD1) in sepsis correlate with survival and ARDS development, thus suggesting plausible novel biomarkers and biological targets for critical illness [48].

8.2.4 Maresins

Maresins are a new family of anti-inflammatory and pro-resolving lipid mediators derived from docosahexaenoic acid (DHA) by macrophages via human 12-lipoxygenase (12-LOX). The first member of this family, termed as maresin 1 (MaR1), exhibits potent phagocyte-directed actions that include inhibition of neutrophil infiltration and stimulation of macrophage efferocytosis by dihydroxyl products in this pathway [10, 49, 50]. In a murine model of ARDS, lipid mediator metabololipidomics discovered that MaR1 was temporally generated and regulated in vivo. Early intravascular MaR1 was organ-protective, and MaR1 production was dependent on platelet-neutrophil interactions leading to reduced lung neutrophils, oedema, tissue hypoxia and inflammatory mediators [51].

Pro-resolution effects are activated to terminate inflammation as soon as the inflammatory response initiates. In a peritonitis model, MaR1 was one of the first SPMs upregulated in the peritoneum during self-resolving infections. Acting as a partial agonist/antagonist to the LTB4 receptor (BLT1), MaR1 supressed the activity of LTB4 so as to promote the uptake and clearance of apoptotic cells and bacteria [52]. Interestingly, levels of MaR1 and LTB4 reached a maximum level in the early stages of the inflammatory response, suggesting that early MaR1 production impacts leukocyte infiltration to the inflammatory site [39, 52].

In a LPS-induced ALI mice model, high dose of MaR1 exhibited protective activities by mitigating patho-histological changes, attenuating pulmonary oedema and restoring oxygenation. Besides, high-dose MaR1 inhibited the increasement of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokines, while anti-inflammatory cytokine IL-10 was upregulated. Moreover, MaR1 lowered LPS-induced neutrophil adhesions and suppressed the expression of intercellular adhesion molecule (ICAM)-1, P-selection and CD24 [53, 54]. Another study reported that MaR1 can maintain the permeability of lung epithelial cells by upregulating the expression of claudin-1 and ZO-1 in LPS-induced ALI [55].

In a CLP sepsis mice model, it was found that MaR1 markedly mitigated the levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6). Intervention of MaR1 lowered the LPS level in serum and enhanced the bacterial clearance. Furthermore, MaR1 attenuated lung injury and decreased serum level of alanine transaminase (ALT), aspartate transaminase (AST), creatinine (Cre) and blood urea nitrogen (BUN) in this sepsis model. Consequently, the survival rate was improved. Inhibition of NF-κB activation pathway by MaR1 is the possible protective mechanism [56].

Maresin 2 (MaR2) was identified later. MaR2 exhibits similar potency to MaR1 in limiting PMN recruitment but has an apparent optimal concentration 2–3 log orders lower than MaR1 in enhancing human macrophage phagocytosis of zymosan. MaR2 also enhanced human macrophage uptake of apoptotic PMN but was less potent than MaR1 [57].

8.2.5 Aspirin-Triggered Lipid Mediators

Aspirin is a classic anti-platelet anti-inflammatory agent. Acetylating COX-2 facilitates aspirin to induce a shift from the synthesis of pro-inflammatory to pro-resolving lipid mediators termed as aspirin-triggered lipoxins (AT-LX) and aspirin-triggered resolvins (AT-Rv) [13, 58]. AT-LX and AT-Rv share the pro-resolution effects of LXA4 and RvD1, respectively, and act by the same intracellular pathways [59].

The aspirin-triggered lipoxin, also known as 15-epi-LXA4 (AT-LXA4), was found to be increased with administration of aspirin in both in vitro and in vivo models of infection; with infection alone increase in 15-epi-LXA4 levels was observed [60]. In murine models of sepsis and ARDS, aspirin increased the survival rate effectively [61]. Low dosage of aspirin administered 30 min prior to the endotoxin model of sepsis improved the survival and reduced the levels of thromboxane and prostaglandins derived from arachidonic acid [62]. Another research indicated that low-dose aspirin, however, did not reduce cytokine and prostaglandin levels while enhancing 15-epi-LXA4 synthesis [63]. In an *E. coli* sepsis model, 15-epi-LXA4 injected 24 h after the injury lowered PMN number in broncho-alveolar lavage by stimulating the apoptosis [64].

Several observational clinical studies have confirmed the benefits of aspirin in those who took it prior to medical consult in sepsis patients. In a group of 1149 critically ill patients, 25% of them with preclinical aspirin use had a decreased risk of

developing to ARDS and tended to a lower mortality [65]. A different study with 5523 patients suffering from systemic inflammatory response syndrome or sepsis suggested that a lower mortality was found in 2082 patients when given aspirin within 24 h after diagnosis [66]. Similarly, in a study comprising 1005 patients with community-acquired pneumonia, 100 mg/day intake of aspirin was associated with a lower mortality rate within 30 days [67]. Hence, a clinical trial indicates that the aspirin and essential fatty acid supplemented in healthy human volunteers increase the endogenous production of pro-resolving mediators including 17-R-PD1 and augment bacterial clearance of leukocytes [68].

However, a multicentre study with 3855 patients did not pinpoint a significant association between preclinical aspirin therapy and progression to ARDS [69]. A prospective observational study with 972 patients indicated that preclinical aspirin therapy was neither associated with the development of organ failure nor shock nor 90-day mortality up to 90 days after hospitalization [70].

Evidence above does not make a solid statement of conclusion on exact beneficial prognoses of aspirin therapy in sepsis. Further studies are required to evaluate a potential protective effect of aspirin in sepsis patients.

8.2.6 Novel Families of SPMs

8.2.6.1 RvTs

A novel family of pro-resolving mediators termed 13 series resolvins (RvTs) were identified recently in the very early stages (≤4 h) of self-resolving *E. coli* infections model. These RvTs mediators include RvT1, RvT2, RvT3 and RvT4, which are derived from n-3 docosapentaenoic acid (n-3 DPA) through sequential reactions involving COX-2/5-LOX in the crosstalk of vascular endothelia and neutrophils. Each of these molecules activates the host immunity in a dose-dependent manner for bacterial clearance and counter-regulates the production of pro-inflammatory molecules including endothelin-1, plasminogen activator inhibitor-1 and inflammatory eicosanoids. Moreover, concentrations of RvTs in peripheral blood increased rapidly after exercise (a self-resolving inflammatory state) in healthy volunteers and were significantly higher in patients with sepsis than in healthy subjects. These results indicate that RvTs biosynthesis occurs in a coordinated manner in correlation with acute activation of the immune response. Therefore, inability to form these pathways leads to delayed resolution responses and an impaired ability to clear bacterial infections [71].

8.2.6.2 PCTRs

Recent studies suggest that the formation of the immunoresolvent protectin conjugate in tissue regenerations (PCTRs: PCTR1, PCTR2 and PCTR3) by group 3 innate lymphoid cells (ILC-3), which in turn regulates peritoneal macrophage responses to bacterial infections, is promoted by the vagus nerve [72]. PCTRs are derived from DHA through 15-LOX-mediated pathways in leukocytes. Since PCTRs actively promote the termination of bacterial infections by stimulating the

uptake and killing the bacteria as well as the repair and regeneration of damaged tissues, these mediators are immunoresolvents [73]. PCTRs parallelly promote a macrophage phenotype shift, downregulating the production of pro-inflammatory cytokines including TNF-α and IL-8 and increase the production of regenerative molecules including TGF-β [74]. Any form of damage to the vagus reflex dysregulates PCTRs formation and macrophage phenotype leading to an impaired ability of the recruited leukocytes to efficiently clear pathogens causing the delayed resolution of inflammatory infections [72].

8.2.6.3 MCTRs

Further researches into mediators formed during the late phases of resolution of *E. coli* infections pointed out a huge group of molecules that are peptide-lipid conjugates. These molecules termed as maresin conjugates in tissue regeneration (MCTRs: MCTR1, MCTR2 and MCTR3) are produced by 14-lipoxygenation of DHA through 12-LOX-mediated pathways in human macrophages. MCTRs exhibit some potential benefits in regulating bacterial phagocytosis, promoting tissue repair and regeneration [74, 75].

Both PCTRs and MCTRs were found in inflammatory exudates and spleens from infected mice as well as in human plasma, serum and spleens. The levels of these mediators were increased during the later stages of infectious inflammation in mice [73, 74].

8.2.7 Carbon Monoxide and Heme Oxygenase

Carbon monoxide (CO) enhances the immune cell function. CO is produced by the constitutively expressed heme oxygenase-2 (HO)-2, and by HO-1, which is upregulated upon cellular stress. HO-1 catalyses the degradation of heme to biliverdin, CO and iron (which binds to ferritin), all acting as anti-oxidative and anti-inflammatory agents [76]. Endogenously formed CO acts as a signalling molecule and induces antioxidant genes [76, 77]. CO downregulates inflammatory prostaglandins and thromboxanes. On the other hand, it enhances the expression of lipoxygenases which are key synthetases of SPMs [15]. Resolvins and lipoxins in turn upregulate HO-1 in macrophages demonstrating mutual amplification of these two pro-resolving pathways [15]. CO contributes to active pathways on the elimination of microorganisms, promote killing of bacteria and enhance their clearance by macrophages in a protective manner [78]. Furthermore, pro-inflammatory cytokines including TLR2, -4, -5 and -9 are repressed, while anti-inflammatory cytokines such as IL-10 are initiated by CO in macrophages [79]. Enhanced endocytosis of apoptotic cells by efferocytosis is also known [15].

In murine sepsis model, higher mortality and hepatic necrosis in HO-1-deficient mice group were found pointing out the protective function of this enzyme [80]. Hepatoprotective effects were reported with increasing levels of CO in endotoxic rats [81]. Hepatic accumulation of PMN, expression of the intercellular adhesion molecule-1 (ICAM-1) and activation of NF-κB in murine polymicrobial sepsis are reduced by CO. In endotoxin-activated human umbilical vein, the endothelial cells

cocultured with CO, the production of reactive oxygen species (ROS), nitric oxide (NO), activation of NF-κB, induction of inducible NO synthase and ICAM-1 and PMN adhesion were reduced [82].

In CLP mice model, levels of circulating inflammatory cytokines and the number of bacteria in blood and organs were decreased by the inhalation of CO which resulted in the increased survival rate of the mice [83]. CO inhalation, 2 h prior to initiation of peritonitis, lowered the number of infiltrating PMN. Monocyte numbers remained unchanged, while the clearance of microbial pathogens and dead PMN significantly raised [15]. Inflammation resolved nearly two times faster than CO nonexposed group. Lipid mediators derived from arachidonic acid, LTB4 and PGE were low in the early stage of inflammation and pro-resolving lipids including RvD1, RvE2 and maresin, were markedly high [15].

The clinical patients have been observed for the protective roles of CO and HO-1. Patients with lower respiratory tract infection indeed had higher CO in their breath, and levels descended in those patients recovering from disease after antibiotic treatment [84]. When compared to 30 healthy neonates, CO in plasma of 7 neonates with sepsis was significantly increased [85]. When 36 patients with severe sepsis or septic shock were compared to 21 patients without sepsis, arterial blood CO and HO-1 protein levels in monocytes were ascended. These two molecules were positively related to survival [86]. Exhaled CO is higher in severely ill patients compared to the control group [87]. In mechanically ventilated patients with severe sepsis or septic shock, exhaled CO was nearly threefold higher compared to controls and declined along with therapy, and high amount of CO in exhaled air on the first day during treatment was associated with better outcome [88]. CO is reported to have beneficial impacts in different patient groups; therefore, inhalation of CO would be a potential policy guide for the treatment of sepsis [77].

8.2.8 Annexin A1

Annexin A1 is a 37 kDa monomeric protein, existing abundantly in some pivotal cell types of the innate and adaptive immune systems and the neuroendocrine system. The synthesis and release of Annexin A1 is regulated by glucocorticoids (GCs) and expresses as a stress response protein through binding to formyl peptide family receptors (FPRs) [89].

An increasing number of experimental evidence in present days indicate that Annexin A1 is crucial to many of the acute actions of GCs in several systems relevant to the stress response, including the innate and adaptive host immune systems [89, 90] and the HPA axis [91]. In Annexin A1-null mice models of inflammation, treating with anti-Annexin A1 antibodies or antisense constructs of Annexin A1, have confirmed that Annexin A1 exhibits as an endogenous regulator of anti-inflammatory and pro-resolving as well as a mediator of GC action [89, 90]. Annexin A1, binding to FPR in the innate immune system, exerts general suppressive activities including the reduction of the amounts of pro-inflammatory eicosanoids molecules generated and the release of histamine and preformed cytokines from mast cells. In contrast, ANX-A1 may induce the release of pro-resolving mediators, such

as IL-10. Furthermore, Annexin A1 highly downregulates PMN migration into inflammatory sites and accelerates their apoptosis, while the migration of monocytes into inflammatory sites is promoted by Annexin A1 which eventually transform the microenvironment into a resolving procedure [92]. In an in vivo experiment, the absence of Annexin A1 or its major receptor (FPR2) amplifies greatly the duration and intensity of acute and chronic inflammation. Consequently, the acute anti-inflammatory actions of GCs are greatly reduced or even abrogated. Conversely, human recombinant Annexin A1 and peptides derived from the N-terminal domain can rescue this phenotype and exert high anti-inflammatory and pro-resolution effects in many models of inflammation [90, 92].

8.3 Summary and Prospect

Sepsis is a severe organ dysfunction syndrome due to uncontrolled infection with a high mortality risk. Despite of inspiring results in preclinical models, none of the presumptive therapeutic agents tested so far succeed in clinical trials. Excessive uncontrolled inflammation and inappropriate immune responses are both characteristic features of sepsis that complicate identification of suitable drug targets. Inhibition of inflammation may delay the resolution because of an improper induction of anti-inflammatory and pro-resolving pathways. Overcoming sepsis, there is still a long way to run.

An accumulating evidence decodes the role that endogenous resolutions triggered by SPMs demonstrate the ensured internal homeostasis in host body by activating the ultimate cardinal signs of resolution that include pathogen and cellular debris lavage, analgesia and restoration of organ functions. Latest studies point out that monitoring the concentrations of these SPMs in infected patients may provide a better understanding of the inflammation-resolution procedures and hence reflect a positive outcome [48]. Yet, requiring further prospective studies to confirm these observations.

The points and the hints that are mentioned in this review indicate that SPMs act as potential biomarkers and, more importantly, hold a great promise as novel therapeutics in inflammatory diseases. Latest studies reveal that the majority of end-stage fatal sepsis patients are immunosuppressed rather than hyperinflammation [93]. Given, the potential anti-inflammatory and pro-resolution benefits of these SPMs (including CO, HO-1 and Annexin A1) mentioned above, endogenous or exogenous supplement of these mediators analogues or natural extracts may be a new paradigm in treating sepsis.

References

1. Singer M. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA. 2016;315(8):801–10.
2. Torio CM. National inpatient hospital costs: the most expensive conditions by payer, 2011. Statistical Brief #160. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. 2013. http://www.ncbi.nlm.nih.gov/books/NBK169005/.

3. Iwashyna TJ. Population burden of long-term survivorship after severe sepsis in older Americans. J Am Geriatr Soc. 2012;60(6):1070–7.
4. Gaieski DF. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med. 2013;41(5):1167–74.
5. Vincent J, ICON Investigators. Assessment of the worldwide burden of critical illness: the Intensive Care Over Nations (ICON) audit. Lancet Respir Med. 2014;2(5):380–6.
6. Fleischmann C, International Forum of Acute Care Trialists. Assessment of global incidence and mortality of hospital-treated sepsis: current estimates and limitations. Am J Respir Crit Care Med. 2016;193(3):259–72.
7. Das UN. Is sepsis a pro-resolution deficiency disorder? Med Hypotheses. 2013;80(3):297–9.
8. Lee CR. Resolvin infectious inflammation by targeting the host response. N Engl J Med. 2015;373(22):2183–5.
9. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510:92–101.
10. Serhan CN. Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta. 2015;1851(4):397–413.
11. Serhan CN. Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. FASEB J. 2017;31(4):1273–88.
12. Dalli J. Does promoting resolution instead of inhibiting inflammation represent the new paradigm in treating infections? Mol Aspects Med. 2017;58:12–20. pii: S0098-2997(17)30021–3.
13. Serhan CN. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med. 2002;196:1025–37.
14. Shinohara M. Novel endogenous proresolving molecules: essential fatty acid-derived and gaseous mediators in the resolution of inflammation. J Atheroscler Thromb. 2016;23(6):655–64.
15. Chiang N. Inhaled carbon monoxide accelerates resolution of inflammation via unique proresolving mediator-heme oxygenase-1 circuits. J Immunol. 2013;190:6378–88.
16. Gobbetti T. Annexin A1 and resolution of inflammation: tissue repairing properties and signalling signature. Biol Chem. 2016;397:981–93.
17. Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins Leukot Essent Fatty Acids. 2005;73:141–62.
18. Serhan CN. Novel anti-inflammatory--pro-resolving mediators and their receptors. Curr Top Med Chem. 2011;11(6):629–47.
19. Psychogios N. The human serum metabolome. PLoS One. 2011;6(2):e16957.
20. Jin SW. Posttreatment with aspirin-triggered lipoxin A4 analog attenuates lipopolysaccharide-induced acute lung injury in mice: the role of heme oxygenase-1. Anesth Analg. 2007;104:369–77.
21. Walker J. Lipoxin A4 increases survival by decreasing systemic inflammation and bacterial load in sepsis. Shock. 2011;36:410–6.
22. Bitto A. Flavocoxid, a dual inhibitor of COX-2 and 5-LOX of natural origin, attenuates the inflammatory response and protects mice from sepsis. Crit Care. 2012;16:R32.
23. Wu B. Lipoxin A4 augments host defense in sepsis and reduces *Pseudomonas aeruginosa* virulence through quorum sensing inhibition. FASEB J. 2016;30:2400–10.
24. Ariel A. Aspirin-triggered lipoxin A4 and B4 analogs block extracellular signal-regulated kinase-dependent TNF-alpha secretion from human T cells. J Immunol. 2003;170:6266–72.
25. Liu H. Effect of BML-111 on the intestinal mucosal barrier in sepsis and its mechanism of action. Mol Med Rep. 2015;12:3101–6.
26. Gobbetti T. Nonredundant protective properties of FPR2/ALX in polymicrobial murine sepsis. Proc Natl Acad Sci U S A. 2014;111:18685–90.
27. Khadaroo RG. ARDS and the multiple organ dysfunction syndrome: common mechanisms of a common systemic process. Crit Care Clin. 2002;18:127–41.
28. Sordi R. Dual role of lipoxin A4 in pneumosepsis pathogenesis. Int Immunopharmacol. 2013;7:283–92.

29. Marshall JC. Why have clinical trials in sepsis failed? Trends Mol Med. 2014;20:195–203.
30. Tsai WH. Plasma levels in sepsis patients of annexin A1, lipoxin A4, macrophage inflammatory protein-3a, and neutrophil gelatinase-associated lipocalin. J Chin Med Assoc. 2013;76:486–90.
31. Serhan CN. Resolvins and protectins in inflammation resolution. Chem Rev. 2011;111:5922–43.
32. Aoki H. Protective effect of resolvin E1 on the development of asthmatic airway inflammation. Biochem Biophys Res Commun. 2010;400(1):128–33.
33. Seki H. The anti-inflammatory and proresolving mediator resolvin E1 protects mice from bacterial pneumonia and acute lung injury. J Immunol. 2010;184:836–43.
34. El Kebir D. Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation. Proc Natl Acad Sci U S A. 2012;109:14983–8.
35. Ariel A. Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. Nat Immunol. 2006;7:1209–16.
36. Lee HN. Resolvin D1-mediated NOX2 inactivation rescues macrophages undertaking efferocytosis from oxidative stress-induced apoptosis. Biochem Pharmacol. 2013;86:759–69.
37. Prieto P. Activation of autophagy in macrophages by pro-resolving lipid mediators. Autophagy. 2015;11:1729–44.
38. Murakami T. Suppressive action of resolvin D1 on the production and release of septic mediators in D-galactosamine-sensitized endotoxin shock mice. Exp Ther Med. 2011;2:57–61.
39. Chiang N. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature. 2012;484:524–8.
40. Kurihara T. Resolvin D2 restores neutrophil directionality and improves survival after burns. FASEB J. 2013;27:2270–81.
41. Chen F. Resolvin D1 improves survival in experimental sepsis through reducing bacterial load and preventing excessive activation of inflammatory response. Eur J Clin Microbiol Infect Dis. 2014;33:457–64.
42. Chen J. Aspirin-triggered resolvin D1 down-regulates inflammatory responses and protects against endotoxin-induced acute kidney injury. Toxicol Appl Pharmacol. 2014;277:118–23.
43. Spite M. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature. 2009;461:1287–91.
44. Park CK. Resolvin D2 is a potent endogenous inhibitor fortransient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. J Neurosci. 2011;31:18433–8.
45. Serhan CN. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol. 2006;176:1848–59.
46. Morita M. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell. 2013;153:112–25.
47. Xia HF. Protectin DX increases survival in a mouse model of sepsis by ameliorating inflammation and modulating macrophage phenotype. Sci Rep. 2017;7:99.
48. Dalli J. Human sepsis eicosanoid and proresolving lipid mediator temporal profiles: correlations with survival and clinical outcomes. Crit Care Med. 2017;45(1):58–68.
49. Serhan CN. Maresins: novel macrophage mediators with potent anti-inflammatory and proresolving actions. J Exp Med. 2009;206:15–23.
50. Sasaki K. Total synthesis and bioactivities of two proposed structures of maresin. Chem Asian J. 2011;6(2):534–43.
51. Abdulnour RE. Maresin 1 biosynthesis during platelet–neutrophil interactions is organ-protective. Proc Natl Acad Sci U S A. 2014;111(46):16526–31.
52. Colas RA. Identification and actions of the maresin 1 metabolome in infectious inflammation. J Immunol. 2016;197(11):4444–52.
53. Gong J. Maresin 1 mitigates LPS-induced acute lung injury in mice. Br J Pharmacol. 2014;171(14):3539–50.
54. Gong J. Maresin 1 prevents lipopolysaccharide-induced neutrophil survival and accelerates resolution of acute lung injury. Shock. 2015;44(4):371–80.

55. Chen L. Maresin 1 maintains the permeability of lung epithelial cells in vitro and in vivo. Inflammation. 2016;39(6):1981–9.
56. Li RD. Maresin 1 mitigates inflammatory response and protects mice from sepsis. Mediators Inflamm. 2016;2016:3798465.
57. Deng B. Maresin biosynthesis and identification of maresin 2, a new anti-inflammatory and pro-resolving mediator from human macrophages. PLoS One. 2014;9(7):e102362.
58. Spite M. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res. 2010;107:1170–84.
59. Rogerio AP. Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. J Immunol. 2012;189:1983–91.
60. Alfredo MB. Protective role of acetylsalicylic acid in experimental Trypanosoma cruzi infection: evidence of a 15-epi-lipoxin A_4-mediated effect. PLoS Negl Trop Dis. 2013;7:e2173.
61. Toner P. Aspirin as a potential treatment in sepsis or acute respiratory distress syndrome. Crit Care. 2015;19:374.
62. Halushka PV. Studies on the beneficial effects of aspirin in endotoxic shock: relationship to inhibition of arachidonic acid metabolism. Am J Med. 1983;74:91–6.
63. Morris T. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol. 2009;183:2089–96.
64. El Kebir D. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med. 2009;180:311–9.
65. Chen W. Prehospital aspirin use is associated with reduced risk of acute respiratory distress syndrome in critically ill patients: a propensity-adjusted analysis. Crit Care Med. 2015;43:801–7.
66. Eisen DP. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. Crit Care Med. 2012;40:1761–7.
67. Falcone M. Lower mortality rate in elderly patients with community-onset pneumonia on treatment with aspirin. J Am Heart Assoc. 2015;4:e001595.
68. Colas RA. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol. 2014;307(1):C39–54.
69. Kor DJ. Association of prehospitalization aspirin therapy and acute lung injury: results of a multicenter international observational study of at-risk patients. Crit Care Med. 2011;39:2393–400.
70. Wiewel MA. Chronic antiplatelet therapy is not associated with alterations in the presentation, outcome, or host response biomarkers during sepsis: a propensity-matched analysis. Intensive Care Med. 2016;42:352–60.
71. Dalli J. Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med. 2015;21(9):1071–5.
72. Dalli J. Vagal regulation of group 3 innate lymphoid cells and the immunoresolvent PCTR1 controls infection resolution. Immunity. 2017;46(1):92–105.
73. Dalli J. Novel proresolving and tissue-regenerative resolvin and protectinsulfido-conjugated pathways. FASEB J. 2015;29(5):2120–36.
74. Ramon S. The protectin PCTR1 is produced by human M2 macrophages and enhances resolution of infectious inflammation. Am J Pathol. 2016;186(4):962–73.
75. Dalli J. Maresin conjugates in tissue regeneration biosynthesis enzymes in human macrophages. Proc Natl Acad Sci U S A. 2016;113(43):12232–7.
76. Sacerdoti D. EETs and HO-1 cross-talk. Prostaglandins Other Lipid Mediat. 2016;125:65–79.
77. Nakahira K. Carbon monoxide in the treatment of sepsis. Am J Physiol Lung Cell Mol Physiol. 2015;309:1387–93.
78. Wegiel B. Macrophages sense and kill bacteria through carbon monoxide-dependent inflammasome activation. J Clin Investig. 2014;124:4926–40.
79. Nakahira K. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. J Exp Med. 2006;203:2377–89.
80. Poss KD. Reduced stress defense in heme oxygenase 1-deficient cells. Proc Natl Acad Sci U S A. 1997;94:10925–30.

81. Kyokane T. Carbon monoxide from heme catabolism protects against hepatobiliary dysfunction in endotoxin-treated rat liver. Gastroenterology. 2001;120:1227–40.
82. Cepinskas G. Carbon monoxide liberated from carbon monoxide-releasing molecule CORM-2 attenuates inflammation in the liver of septic mice. Am J Physiol Gastrointest Liver Physiol. 2008;294:184–91.
83. Lee S. Carbon monoxide confers protection in sepsis by enhancing beclin 1-dependent autophagy and phagocytosis. Antioxid Redox Signal. 2014;20:432–42.
84. Biernacki WA. Exhaled carbon monoxide in patients with lower respiratory tract infection. Respir Med. 2001;95:1003–5.
85. Shi Y. Plasma carbon monoxide levels in term newborn infants with sepsis. Biol Neonate. 2000;78:230–2.
86. Takaki S. Beneficial effects of the heme oxygenase-1/carbon monoxide system in patients with severe sepsis/septic shock. Intensive Care Med. 2010;36:42–8.
87. Morimatsu H. Increased heme catabolism in critically ill patients: correlation among exhaled carbon monoxide, arterial carboxyhemoglobin, and serum bilirubin IX alpha concentrations. Am J Physiol Lung Cell Mol Physiol. 2006;290:114–9.
88. Zegdi R. Increased endogenous carbon monoxide production in severe sepsis. Intensive Care Med. 2002;28:793–6.
89. D'Acquisto F. Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. Br J Pharmacol. 2008;155(2):152–69.
90. Perretti M. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol. 2009;9(1):62–70.
91. Buckingham JC. Annexin 1, glucocorticoids, and the neuroendocrine-immune interface. Ann N Y Acad Sci. 2006;1088:396–409.
92. Perretti M. Annexin I is stored within gelatinase granules of human neutrophil and mobilized on the cell surface upon adhesion but not phagocytosis. Cell Biol Int. 2000;24(3):163–74.
93. Hotchkiss RS. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013;13:862–74.

Novel Insights into Anti-inflammatory Therapy in Sepsis-Induced ARDS

9

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Abstract

Acute respiratory distress syndrome (ARDS) is a life-threatening complication caused by diverse conditions such as sepsis, trauma, and so on. Despite recent advances in understanding and management of ARDS, the mortality rate of ARDS remains high. Given the complex dysregulated inflammatory cascade in ARDS, there is an increasing interest in anti-inflammatory therapy targeted at improving the outcome of ARDS. This review will summarize current and future direction of anti-inflammatory therapy in ARDS.

Keywords

Acute respiratory distress syndrome · Sepsis · Anti-inflammatory therapy

9.1 Sepsis

Sepsis is one of the oldest and most intricate syndromes in medicine. It is the most common etiology of ARDS and is associated with the highest case-fatality rate [1–3]. Sepsis means "rotting" or "decaying." The word "sepsis" (σήψις) is derived from the ancient Greek literary work Homer's epic. With the establishment of germ theory by Pasteur in the nineteenth century, people realized that sepsis was the result of the invasion of host by pathogenic organisms that then spread in the bloodstream [4]. In 1991, an international consensus conference defined sepsis as host's systemic inflammatory response syndrome (SIRS) to infection. Sepsis complicated with acute organ dysfunction was termed severe sepsis. "Septic shock" was defined as refractory hypotension induced by sepsis despite adequate

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fluid resuscitation. Since then, the concept of SIRS has been globally adopted by the clinicians and investigators. In the second international conference of sepsis definition in 2001, participants endorsed most of these concepts and expanded the diagnostic criteria [2]. However, the new version was scarcely used in clinical practice due to its complexity and lack of supporting evidence. Recently in 2016, definition of sepsis was updated. The third international consensus conference defined sepsis as a life-threatening organ dysfunction caused by dysregulated host response to infection. The task force recommended using an acute change in total SOFA scores >2 to represent organ dysfunction. Septic shock was defined as a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. The definition and diagnostic criteria of sepsis had been updated several times, which reflects the intricacy of sepsis and the importance of clinical diagnosis and treatment. Although methods of monitoring and assessment for sepsis have been developed, the current sepsis treatment strategy is still not optimistic. It is estimated that sepsis is one of the leading causes of mortality and critical illness worldwide, which has posed a huge challenge for clinicians.

Sepsis is a generalized inflammatory syndrome of the host in response to various infectious stimuli [3]. Vascular inflammation during sepsis development is characterized by activation and recruitment of immune cells to sites of inflammation where they are needed to combat exogenous pathogens [4]. This process increases the permeability of vascular endothelium, leading to excessive fluid loss from the intravascular space. The resulted tissue edema also contributes to the development of organ dysfunction, including acute respiratory distress syndrome (ARDS) of the lung.

9.2 Acute Respiratory Distress Syndrome (ARDS)

Acute respiratory distress syndrome (ARDS) is a life-threatening respiratory condition characterized by hypoxemia and decreased pulmonary compliance. It is an acute respiratory failure that can be caused by a variety of insults. The pathophysiology varies and depends on the insult type. ARDS and sepsis have similar underlying mechanisms: dysregulated inflammation and endothelial dysfunction (which leads to alveolar barrier disruption in ARDS). The pathological features of sepsis-induced ARDS include damage of alveolar epithelium and capillary endothelium, inflammatory cell infiltration, and pulmonary edema. Disruption of alveolar barrier results in increased permeability to water, proteins, and other solutes. Intra-alveolar accumulation of leukocytes and erythrocytes is also associated with altered endothelial and epithelial function. Disruption of VE-cadherin bonds is a central mechanism of altered endothelial barrier function in both experimental acute lung injury (ALI) and models of sepsis and systemic vascular destabilization [5]. Increased permeability of microvascular barriers, which causes extravascular accumulation of protein-rich fluid, is a cardinal feature of acute inflammation and a central pathophysiologic mechanism in ARDS.

Resolution of ARDS requires removal of alveolar edema fluid, removal of the acute inflammatory cells, and repair of the injured alveolar epithelium. Resolution of inflammation in ARDS requires the removal of neutrophils from the distal airspace of the lung. Restoration of the alveolar epithelial barrier requires reepithelialization by proliferation of alveolar type II cells [6].

Many clinical trials have assessed pharmacologic interventions, innovative strategies for positive-pressure ventilation, and other supportive approaches to ARDS treatment. However, no single treatment has been testified to reverse the pathological process of ARDS. Integrated therapy combining several supporting strategies can possibly improve the outcome. Among the generic therapy, anti-inflammatory therapy aiming at blocking the uncontrolled inflammation in sepsis-induced ARDS may be a promising intervention. In this chapter, we focus on the existing and potential anti-inflammatory therapy in sepsis-induced ARDS.

9.2.1 Glucocorticoids

Glucocorticoids are widely used to reduce inflammation. The major mechanism is to suppress the expression of cytokine-induced genes by binding to the cytoplasmic steroid receptor. Considering the anti-inflammatory effect of glucocorticoids, it may benefit patients with sepsis-induced ARDS. However, clinical trials exhibited contradictory results. Four trials of high-dose, short course methylprednisolone for early-phase ARDS had shown no significant benefit on survival [7–10]. In fibroproliferative phase of ARDS, treatment with prolonged high-dose corticosteroids was shown to be beneficial [11–13]. A clinical trial with small sample size discovered that prolonged use of moderate-dose methylprednisolone in unresolving ARDS not only ameliorated lung injury and MODS scores but also reduced mortality [14]. In addition, prolonged methylprednisolone administration was shown to be effective in accelerating the resolution peripheral acquired glucocorticoid resistance in ARDS [15]. However, results from a multicenter RCT did not support routine use of moderate-dose methylprednisolone in persistent ARDS. A post hoc analysis showed treatment with low dose of corticosteroids was associated with better outcomes in septic shock-associated early ARDS and a weak cortisol response to corticotropin [16]. Similarly, Meduri et al. identified that prolonged low-dose infusion of methylprednisolone in early severe ARDS was associated with significant improvement in pulmonary and extrapulmonary organ dysfunction [17]. Nonetheless, several studies with larger sample size confirmed the futility of corticosteroids use in ARDS patients [18–20]. In sepsis-induced ARDS, hydrocortisone treatment was associated with a significant improvement in pulmonary physiology, but has no significant survival benefit [21]. In a word, the function of corticosteroids in ARDS is still uncertain. Even so, physicians are still prescribing corticosteroids as an alternative to conventional therapy when treating patients with ARDS. As is well known, the side effects of corticosteroids include hyperglycemia, poor wound healing, psychosis, pancreatitis, and prolonged muscle weakness with impaired functional status [22]. Several studies suggested high-dose corticosteroids use in sepsis-induced

ARDS increased risk of secondary infections [8, 10, 23]. Moderate-dose corticosteroids for sepsis yet did not increase the risk of adverse events, which was supported by a systematic analysis [24]. Recently, the 2016 SCC (Surviving Sepsis Campaign) guidelines suggested intravenous hydrocortisone at a dose of 200 mg/day if hemodynamic stability is unachievable despite adequate fluid resuscitation [25]. The 2016 Japanese clinical guideline for management of ARDS recommended the administration of steroids equivalent to methylprednisolone 1–2 mg/kg/day [26].

9.2.2 Statins

Statins (3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors) is a class of therapeutics that has been used broadly in treating cardiovascular diseases. The primary mechanism is to reduce low-density lipoprotein cholesterol levels. Accumulating evidence suggests that statins have anti-inflammatory properties which may broaden their use outside cardiovascular system [27]. The anti-inflammatory property of statins differs from one another, but the potency in lowing cholesterol is similar. To be specific, cerivastatin, atorvastatin, and simvastatin have more anti-inflammatory effects than fluvastatin, lovastatin, or pravastatin [28]. Statins can be classified into hydrophilic statins (e.g., pravastatin, rosuvastatin, etc.) and hydrophobic statins (e.g., cerivastatin, fluvastatin, atorvastatin, simvastatin, etc.). Evidence from animal experiments indicated that simvastatin may improve the outcome of acute lung injury, which formed the basis for the subsequent human trials [29]. The role of statins in patients with sepsis and ARDS is now under heated debate [30–32]. Initial observational studies showed significant reduction in sepsis mortality with statin use, which was consistent with the results of animal experiments [27, 33]. However, the author recognized the limitations of high heterogeneity and possible bias in the data and called for cautious use of statins in sepsis. It is not surprising that subsequent randomized clinical trials (RCTs) using atorvastatin or simvastatin in septic patients showed no improvement in clinical outcome [34]. The largest trial (SAILS) to date evaluated adjunctive rosuvastatin therapy in sepsis-induced ARDS. However, this trial was forced to terminate due to failing to demonstrate 60-day morality benefit [35]. In an individual patient data meta-analysis, Gordon et al. found no clinical benefit from initiation of statin therapy in adult patients with ARDS, either overall or in predefined subgroups classified by dosage or lipo/hydrophilic properties [36]. Collectively, at the present time, routine use of statins in sepsis or ARDS is not recommended. Future studies should focus on identifying targeted patients, more effective agent, and appropriate dosing with optimized trial methodology. The potential of statins in preventing ARDS in septic patients needs to be evaluated.

9.2.3 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Aspirin belongs to NSAIDs and is a nonselective inhibitor of the enzyme cyclooxygenase (COX). High dose of aspirin has been used for the treatment of rheumatic

fever. Low dose of aspirin is now utilized for the primary and secondary prevention of cardiovascular diseases. Recently, aspirin has been indicated to act a potential role in preventing and treating sepsis and ARDS. There are several mechanisms of aspirin that can ameliorate sepsis and/or ARDS: (1) inhibition of COX, (2) inhibition of nuclear factor kappa B (NF-κB), (3) production of nitric oxide (NO), and (4) lipoxin production [37]. Preclinical evidence from sepsis and ARDS animal model suggested that aspirin can reduce lung injury and mortality [38]. Several observational studies demonstrated that aspirin can decrease mortality of the septic shock or ARDS patients in ICU [39–41]. Furthermore, a recent systemic review of preclinical studies and meta-analysis of clinical studies suggested a beneficial role for aspirin in prevention and treatment of ARDS [42]. A prospective cohort study assessed the association between prehospital statin and aspirin use and the prevalence of severe sepsis and ALI/ARDS; although there was no significant statistical difference, patients with prehospital aspirin combined with statin treatment had the lowest rate of sepsis or ARDS [43]. On the contrary, a multicenter analysis found no significant association between prehospital aspirin use and development of ARDS [44]. Furthermore, result from a RCT enrolling at-risk patients from emergency department showed that aspirin did not reduce the risk of ARDS at 7 days [45]. Additionally, a large RCT examined the role of ibuprofen in sepsis and there was no improvement in mortality [46]. Aspirin treatment increases the risk of bleeding. The safety of aspirin in patients with thrombocytopenia, which is commonly seen in sepsis and ARDS, has not been studied in prospective RCTs. Several clinical trials are still in progress exploring aspirin's role in prevention and treatment of sepsis and ARDS [47].

9.2.4 Urinary Trypsin Inhibitor

Urinary trypsin inhibitor, also referred to as ulinastatin or bikunin, is a selective serine protease inhibitor derived from human urine. It has strong anti-inflammatory and anticoagulant activity. It is cleaved from the larger inter-a-trypsin inhibitor molecule by neutrophil elastase in the presence of inflammation [48]. In animal experiments of sepsis, ulinastatin ameliorated systemic inflammation and organ dysfunction [49–51]. The usefulness of ulinastatin in sepsis-induced ARDS was first examined by Japanese researchers. Their results suggested ulinastatin could improve the prognosis of sepsis-induced ARDS [52]. Several clinical studies have testified that ulinastatin can reduce mortality in patients with severe sepsis [53]. However, the more widespread use of ulinastatin requires high-quality clinical trials in non-Asian population and a standard dosing guideline as well.

9.2.5 Immunomodulatory Therapies

Much progress has been made in our understanding of the pathophysiology of sepsis-induced ARDS. As a common etiology of ARDS, sepsis is caused by

uncontrolled host-pathogen defense. The pro-inflammatory and anti-inflammatory processes are simultaneously activated at the onset of sepsis. To be more accurate, an immune suppression phase follows the initial hyperinflammation phase. Based on the biphasic theory, several anti-cytokine therapies targeting the pro-inflammation phase have been investigated, such as tumor necrosis factor(TNF)-α inhibitor and interleukin(IL)-1 receptor antagonist. Inhibition of TNF-α and IL-1 has been shown to decrease sepsis mortality in preclinical studies, but these agents failed to show similar effect in clinical trials [54]. It seems that blocking a single cytokine may not halt the noxious inflammatory response. In addition, TNF-α and IL-1, which are early inflammatory mediators in sepsis, may have reached peak concentration before targeted medical intervention. Therefore, targeting late inflammatory mediators of sepsis becomes a logical alternative. However, these late mediators (high-mobility group B-1 (HMGB1), histones, etc.), which contribute to organ injury in sepsis, need to be assessed in human sepsis trials [55]. The activation of innate immune system generates free radicals. In sepsis, free radicals kill the pathogens but also cause tissue injury and cellular dysfunction. Several antioxidants such as N-acetylcysteine have been investigated in consideration of their potency in restoration of the redox balance. Despite the promise in preclinical studies, antioxidant therapy in human trials failed to show consistent benefits in sepsis [54]. As more patients survive the pro-inflammation phase of sepsis, the adverse consequences of immune suppression phase become apparent. Thus, several agents (e.g., exogenous interferon (IFN)-γ, IL-7) were designed to promote immune response in sepsis. Three RCTs showed that IFN-γ treatment reduced the number of infection-related deaths in severely injured patients. There is another ongoing clinical trial assessing the effects of adjunctive therapy with IFN-γ on immune functions in septic patients (Clinicaltrials.gov # NCT01649921). Several preclinical studies indicated that IL-7 has significant beneficial effects in sepsis. The clinical trial evaluating the beneficial effect of IL-7 in septic patients (registered on ClinicalTrials.gov # NCT02640807) is still in progress [56]. Overall, immunomodulatory therapies may have promising potential in treating sepsis-induced ARDS.

9.2.6 Continuous Renal Replacement Therapy (CRRT)

Based on the biphasic theory of hyperinflammation and immune suppression in sepsis, CRRT might be beneficial in restoring immunohomeostasis through removing both pro- and anti-inflammatory mediators continuously and nonspecifically. However, the capability of CRRT to remove inflammatory mediators remains controversial. Some animal and human studies have shown that CRRT can extract nearly every substance involved in sepsis to a certain extent [57]. CRRT was manifested to remove pro-inflammatory cytokines (e.g., macrophage migration inhibitory factor (MIF) and HMGB1) in sepsis patients. On the contrary, some studies showed that CRRT can only remove insubstantial number of mediators compared with endogenous clearance [58]. Although the decrease of plasma cytokine levels is unmeasurable, significant clinical benefits have been observed in terms of

CRRT. This makes the measurement of plasma cytokine levels debatable. Since inflammation in sepsis is caused by a network of interdependent mediators, even a small decrease of cytokine levels can induce significant balance changes. Furthermore, CRRT can positively influence the outcome of sepsis in other ways. The 2016 International Guidelines for Management of Sepsis and Septic Shock suggests using CRRT to facilitate management of fluid balance in hemodynamically unstable septic patients [25]. However, large multicenter studies are needed to make further recommendations as to the optimal use of CRRT in sepsis-induced ARDS.

9.3 Future Direction

In the past few decades, major progress has been made in reducing mortality of ARDS, including lung-protective ventilation, neuromuscular blockade, fluid conservative therapy, etc. Nonetheless, the lack of effective pharmacological therapies for ARDS remains a challenge in the field. Ineffective pharmacological interventions in treating ARDS include inhaled vasodilators, β2 agonists, antioxidants, lisophylline, prostaglandin E1, neutrophil elastase inhibitors, activated protein C, ketoconazole, and surfactant [59]. Most of them were once being considered to develop into generic therapies in sepsis and ARDS due to their anti-inflammatory characteristics. Apart from the anti-inflammatory drugs mentioned above, other generic therapies, including paracetamol, azithromycin, and vitamin C supplementation, have been suggested promising roles in treating sepsis and ARDS considering their potential anti-inflammatory properties. However, these anti-inflammatory therapies, which were testified to be effective in preventing or treating lung injury in preclinical studies, were less effective or ineffective in the RCTs. The disappointing results of RCTs might be attributed to the heterogeneity of enrolled ARDS patients. Improvement of homogeneity in ARDS clinical research may contribute to discovering possible benefit of a specific therapy (e.g., the trials of prone positioning and neuromuscular blockade for ARDS) [60, 61]. Besides, optimizing and innovative trail designs are essential for developing future therapies in ARDS. For example, adaptive trail designs which provide more flexibility in regard to power and effect size compared with traditional RCTs are recommended to test new therapies for ARDS. The promising preclinical data supports the role of anti-inflammatory drugs in the prevention of ARDS. The National Heart Lung Blood Institute (NHLBI) has advocated the development of strategies to perform ALI prevention trials [62]. The results of ongoing clinical trials regarding anti-inflammatory therapy for treating or preventing ARDS will further elucidate their role in this field.

Other new therapies including endothelium and late molecular mediators of septic organ injury merit thorough investigation. Endothelial injury during sepsis leads to increased endothelial permeability, which contributes to edema in diverse organs. Based on this theory, therapies targeting endothelium seem appealing. In animal models of sepsis, several biological molecules (angiopoietin-1 (Ang1), Slit2N, sphingosine-1-phosphate (S1P)) have been demonstrated to alter endothelial cell permeability through targeting endothelial tight junctions [55]. The endothelial

glycocalyx which is highly relevant to septic organ injury and microvascular dysfunction is another potential target. Reinforcement of endothelial barrier function is suggested to alleviate vascular leak, which will apparently benefit patients of sepsis or ARDS. However, general endothelium-protective interventions failed to improve clinical outcomes. Endothelium-protective interventions might only benefit patients who exhibit baseline abnormalities of endothelial function. Therefore, future studies will pursue endothelium-protective, "personalized" approaches in terms of rational detection of systemic endothelial damage markers. Late mediators (HMGB1, histones, etc.) of septic inflammation have been shown to deteriorate late endothelial barrier dysfunction [55]. Blocking the action of these mediators with specific antibodies can improve survival in animal experiments. However, application of these new therapies still requires assessment in human clinical trials.

References

1. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. N Engl J Med. 2005;353(16):1685–93.
2. Hudson LD, Steinberg KP. Epidemiology of acute lung injury and ARDS. Chest. 1999;116(1 Suppl):74s–82s.
3. Stapleton RD, Wang BM, Hudson LD, Rubenfeld GD, Caldwell ES, Steinberg KP. Causes and timing of death in patients with ARDS. Chest. 2005;128(2):525–32.
4. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013;369(9):840–51.
5. Vestweber D, Winderlich M, Cagna G, Nottebaum AF. Cell adhesion dynamics at endothelial junctions: VE-cadherin as a major player. Trends Cell Biol. 2009;19(1):8–15.
6. Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. J Clin Invest. 2012;122(8):2731–40.
7. Bone RC, Fisher CJ Jr, Clemmer TP, Slotman GJ, Metz CA. Early methylprednisolone treatment for septic syndrome and the adult respiratory distress syndrome. Chest. 1987;92(6):1032–6.
8. Weigelt JA, Norcross JF, Borman KR, Snyder WH 3rd. Early steroid therapy for respiratory failure. Arch Surg. 1985;120(5):536–40.
9. Luce JM, Montgomery AB, Marks JD, Turner J, Metz CA, Murray JF. Ineffectiveness of high-dose methylprednisolone in preventing parenchymal lung injury and improving mortality in patients with septic shock. Am Rev Respir Dis. 1988;138(1):62–8.
10. Bernard GR, Luce JM, Sprung CL, et al. High-dose corticosteroids in patients with the adult respiratory distress syndrome. N Engl J Med. 1987;317(25):1565–70.
11. Keel JB, Hauser M, Stocker R, Baumann PC, Speich R. Established acute respiratory distress syndrome: benefit of corticosteroid rescue therapy. Respiration. 1998;65(4):258–64.
12. Meduri GU, Belenchia JM, Estes RJ, Wunderink RG, el Torky M, Leeper KV Jr. Fibroproliferative phase of ARDS. Clinical findings and effects of corticosteroids. Chest. 1991;100(4):943–52.
13. Biffl WL, Moore FA, Moore EE, Haenel JB, McIntyre RC Jr, Burch JM. Are corticosteroids salvage therapy for refractory acute respiratory distress syndrome? Am J Surg. 1995;170(6):591–5; discussion 595–596.
14. Meduri GU, Headley AS, Golden E, et al. Effect of prolonged methylprednisolone therapy in unresolving acute respiratory distress syndrome: a randomized controlled trial. JAMA. 1998;280(2):159–65.
15. Meduri GU, Tolley EA, Chrousos GP, Stentz F. Prolonged methylprednisolone treatment suppresses systemic inflammation in patients with unresolving acute respiratory distress syndrome: evidence for inadequate endogenous glucocorticoid secretion and inflammation-induced immune cell resistance to glucocorticoids. Am J Respir Crit Care Med. 2002;165(7):983–91.

16. Annane D, Sebille V, Bellissant E. Effect of low doses of corticosteroids in septic shock patients with or without early acute respiratory distress syndrome. Crit Care Med. 2006;34(1):22–30.
17. Meduri GU, Golden E, Freire AX, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest. 2007;131(4):954–63.
18. Zhang Z, Chen L, Ni H. The effectiveness of corticosteroids on mortality in patients with acute respiratory distress syndrome or acute lung injury: a secondary analysis. Sci Rep. 2015;5:17654.
19. Steinberg KP, Hudson LD, Goodman RB, et al. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med. 2006;354(16):1671–84.
20. Peter JV, John P, Graham PL, Moran JL, George IA, Bersten A. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. BMJ. 2008;336(7651):1006–9.
21. Tongyoo S, Permpikul C, Mongkolpun W, et al. Hydrocortisone treatment in early sepsis-associated acute respiratory distress syndrome: results of a randomized controlled trial. Crit Care. 2016;20(1):329.
22. Herridge MS, Cheung AM, Tansey CM, et al. One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med. 2003;348(8):683–93.
23. Bone RC, Fisher CJ Jr, Clemmer TP, Slotman GJ, Metz CA, Balk RA. A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N Engl J Med. 1987;317(11):653–8.
24. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. BMJ. 2004;329(7464):480.
25. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit Care Med. 2017;45(3):486–552.
26. Hashimoto S, Sanui M, Egi M, et al. The clinical practice guideline for the management of ARDS in Japan. J Intensive Care. 2017;5:50.
27. Dinarello CA. Anti-inflammatory agents: present and future. Cell. 2010;140(6):935–50.
28. Hilgendorff A, Muth H, Parviz B, et al. Statins differ in their ability to block NF-kappaB activation in human blood monocytes. Int J Clin Pharmacol Ther. 2003;41(9):397–401.
29. Jacobson JR, Barnard JW, Grigoryev DN, Ma SF, Tuder RM, Garcia JG. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. Am J Physiol Lung Cell Mol Physiol. 2005;288(6):L1026–32.
30. Kruger PS, Terblanche M. Statins in patients with sepsis and ARDS: is it over? No. Intensive Care Med. 2017;43(5):675–6.
31. Alhazzani W, Truwit J. Statins in patients with sepsis and ARDS: is it over? Yes. Intensive Care Med. 2017;43(5):672–4.
32. McAuley D, Charles PE, Papazian L. Statins in patients with sepsis and ARDS: is it over? We are not sure. Intensive Care Med. 2017;43(5):677–9.
33. Wan YD, Sun TW, Kan QC, Guan FX, Zhang SG. Effect of statin therapy on mortality from infection and sepsis: a meta-analysis of randomized and observational studies. Crit Care. 2014;18(2):R71.
34. Pasin L, Landoni G, Castro ML, et al. The effect of statins on mortality in septic patients: a meta-analysis of randomized controlled trials. PLoS One. 2013;8(12):e82775.
35. Truwit JD, Bernard GR, Steingrub J, et al. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. N Engl J Med. 2014;370(23):2191–200.
36. Nagendran M, McAuley DF, Kruger PS, et al. Statin therapy for acute respiratory distress syndrome: an individual patient data meta-analysis of randomised clinical trials. Intensive Care Med. 2017;43(5):663–71.
37. Toner P, McAuley DF, Shyamsundar M. Aspirin as a potential treatment in sepsis or acute respiratory distress syndrome. Crit Care. 2015;19:374.
38. Looney MR, Nguyen JX, Hu Y, Van Ziffle JA, Lowell CA, Matthay MA. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. J Clin Invest. 2009;119(11):3450–61.

39. Eisen DP, Reid D, McBryde ES. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. Crit Care Med. 2012;40(6):1761–7.
40. Sossdorf M, Otto GP, Boettel J, Winning J, Losche W. Benefit of low-dose aspirin and non-steroidal anti-inflammatory drugs in septic patients. Crit Care. 2013;17(1):402.
41. Boyle AJ, di Gangi S, Hamid UI, et al. Aspirin therapy in patients with acute respiratory distress syndrome (ARDS) is associated with reduced intensive care unit mortality: a prospective analysis. Crit Care. 2015;19:109.
42. Panka BA, de Grooth HJ, Spoelstra-de Man AM, Looney MR, Tuinman PR. Prevention or treatment of ards with aspirin: a review of preclinical models and meta-analysis of clinical studies. Shock. 2017;47(1):13–21.
43. O'Neal HR Jr, Koyama T, Koehler EA, et al. Prehospital statin and aspirin use and the prevalence of severe sepsis and acute lung injury/acute respiratory distress syndrome. Crit Care Med. 2011;39(6):1343–50.
44. Kor DJ, Erlich J, Gong MN, et al. Association of prehospitalization aspirin therapy and acute lung injury: results of a multicenter international observational study of at-risk patients. Crit Care Med. 2011;39(11):2393–400.
45. Kor DJ, Carter RE, Park PK, et al. Effect of aspirin on development of ARDS in at-risk patients presenting to the emergency department: the LIPS-A randomized clinical trial. JAMA. 2016;315(22):2406–14.
46. Bernard GR, Wheeler AP, Russell JA, et al. The effects of ibuprofen on the physiology and survival of patients with sepsis. The Ibuprofen in Sepsis Study Group. N Engl J Med. 1997;336(13):912–8.
47. Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. Lancet Respir Med. 2017;5(6):524–34.
48. Pugia MJ, Lott JA. Pathophysiology and diagnostic value of urinary trypsin inhibitors. Clin Chem Lab Med. 2005;43(1):1–16.
49. Wakahara K, Kobayashi H, Yagyu T, et al. Bikunin suppresses lipopolysaccharide-induced lethality through down-regulation of tumor necrosis factor- alpha and interleukin-1 beta in macrophages. J Infect Dis. 2005;191(6):930–8.
50. Ueki M, Taie S, Chujo K, et al. Urinary trypsin inhibitor reduces inflammatory response in kidney induced by lipopolysaccharide. J Biosci Bioeng. 2007;104(4):315–20.
51. Cao YZ, Tu YY, Chen X, Wang BL, Zhong YX, Liu MH. Protective effect of Ulinastatin against murine models of sepsis: inhibition of TNF-alpha and IL-6 and augmentation of IL-10 and IL-13. Exp Toxicol Pathol. 2012;64(6):543–7.
52. Kawai S, Sakayori S, Watanabe H, Kobayashi H. Usefulness of a protease inhibitor (urinastatin) in ARDS with infectious diseases. Nihon Kyobu Shikkan Gakkai Zasshi. 1990;28(6):843–51.
53. Karnad DR, Bhadade R, Verma PK, et al. Intravenous administration of ulinastatin (human urinary trypsin inhibitor) in severe sepsis: a multicenter randomized controlled study. Intensive Care Med. 2014;40(6):830–8.
54. Yadav H, Cartin-Ceba R. Balance between hyperinflammation and immunosuppression in sepsis. Semin Respir Crit Care Med. 2016;37(1):42–50.
55. Seeley EJ, Bernard GR. Therapeutic targets in sepsis: past, present, and future. Clin Chest Med. 2016;37(2):181–9.
56. Patil NK, Bohannon JK, Sherwood ER. Immunotherapy: a promising approach to reverse sepsis-induced immunosuppression. Pharmacol Res. 2016;111:688–702.
57. de Vriese AS, Vanholder RC, Pascual M, Lameire NH, Colardyn FA. Can inflammatory cytokines be removed efficiently by continuous renal replacement therapies? Intensive Care Med. 1999;25(9):903–10.
58. Sieberth HG, Kierdorf HP. Is cytokine removal by continuous hemofiltration feasible? Kidney Int Suppl. 1999;72:S79–83.
59. Yadav H, Thompson BT, Gajic O. Fifty years of research in ARDS. Is acute respiratory distress syndrome a preventable disease? Am J Respir Crit Care Med. 2017;195(6):725–36.

60. Papazian L, Forel JM, Gacouin A, et al. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med. 2010;363(12):1107–16.
61. Guerin C, Reignier J, Richard JC, et al. Prone positioning in severe acute respiratory distress syndrome. N Engl J Med. 2013;368(23):2159–68.
62. Spragg RG, Bernard GR, Checkley W, et al. Beyond mortality: future clinical research in acute lung injury. Am J Respir Crit Care Med. 2010;181(10):1121–7.

Surviving Sepsis: Tolerance Towards Bacteria and Their Cell Wall Components

10

Ming-Sheng Lim, H. Paul Redmond, and Jianghuai Wang

Abstract

Trauma and surgery are intricately linked and activate the inflammatory response, which may be systemic especially in the setting of superimposed sepsis. While the inflammatory response developed evolutionarily as a mechanism to survive sepsis, it is now known that an excessive systemic response is the primary determinant leading to a poor outcome in patients with sepsis. The systemic inflammatory response is triggered in turn by recognition of molecular patterns, either from invasive pathogens or from tissue injury, by inflammatory cells or leucocytes such as macrophages and monocytes, which sets off a chain or cascade of molecular events that ultimately result in the production and release of proinflammatory cytokines, chemokines and other factors. This process is like a double-edged sword—on the one hand, innate immune cells are recruited to oppose invading pathogens, and factors required for wound healing are recruited to the site of injury; on the other hand, if an excessive response occurs, inflammatory mediators can result in local and systemic tissue injury leading to multi-organ dysfunction and death. Tolerance describes an interesting phenomenon whereby following an initial sensitising event where exposure of innate immune cells to immune-stimulating agents results in the expected inflammatory response, further, repeated exposure of immune-stimulating agents results in a reduced and dampened response. The implication is that there exists a built-in regulatory mechanism to prevent the excessive inflammatory response that is responsible for the bulk of the morbidity and mortality in sepsis. Understanding the molecular pathways involved in tolerance could allow the potential manipulation, which, if successful, could translate into better care and outcomes for patients suffering from the systemic inflammatory response syndrome following trauma, surgery or sepsis.

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10.1 Introduction

Trauma and surgery are inseparable. Surgery is often required following major trauma, and surgery by its very nature causes trauma to the body. Both trauma and surgery trigger the inflammatory response, which may be either localised at the wound site or systemic. The latter is seen especially following large surgical procedures, if the degree of trauma is extensive, or in the setting of sepsis which is a common clinical complication shared by both.

Sepsis is defined as life-threatening organ dysfunction due to a dysregulated host response to infection [1]. In the USA, cases of severe sepsis rose from 415,280 in 2003 to 711,736 in 2007, with a mortality rate of almost 30% in 2007 [2]. A systematic review comparing sepsis in the burn, trauma and general intensive care unit patients found that the incidence of sepsis reached 17% in trauma and 43% for burns, with mortality rates of up to 37% and 65%, respectively [3]. While sepsis is comparatively rarer in the elective surgical setting, with a retrospective review reporting sepsis in only 2% of 229,918 patients, the risk of mortality remains high at 20–26% [4].

The morbidity and mortality associated with sepsis is largely a result of organ dysfunction as demonstrated by the Sepsis-related Organ Failure Assessment (SOFA) score [5]. This is often caused by the host inflammatory response to pathogens, rather than due to direct, pathogen-mediated cytotoxicity [6]. The inflammatory response in sepsis, the pathways involved in its regulation, the protective effects of immune tolerance and how they may be manipulated to the benefit of patients form the basis of this chapter.

10.2 The Double-Edged Sword of Inflammation

The discovery that the excessive host inflammatory response is the primary determinant leading to a poor outcome in sepsis is relatively recent. Despite the global disease burden, sepsis only received a formal definition from the American College of Chest Physicians and Society of Critical Care consensus meeting in 1992, with updates in 2001 and 2016 [1, 7, 8]. The previous definition of sepsis required the clinical manifestations of the systemic inflammatory response syndrome (SIRS) due to a presumed infective source, and the latest definition of sepsis emphasises the dysregulated host response to infection [1].

Inflammation is a double edged sword: It causes harm in excess and dysregulated, but is an essential component of the body's response to infection and trauma, a well recognised fact since ancient times (Fig. 10.1). Hippocrates in the fifth century BC coined the term oedema to describe the swelling seen in inflammation. The

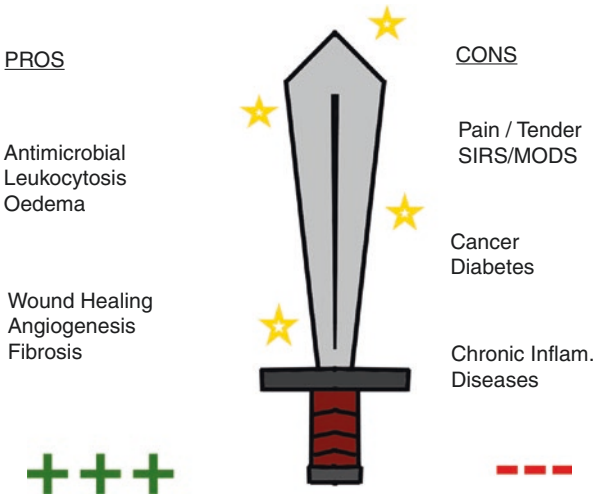

Fig. 10.1 The double-edged sword of inflammation. Inflammation is undeniably central to essential bodily functions, such as the response towards pathogens, stress and trauma (left), but is associated with considerable morbidity and mortality and is part of the pathophysiology of multiple other diseases (right)

roman writer Aulus Celsus later described redness, warmth, swelling and pain as the four main signs of inflammation. Galen, physician and surgeon to Marcus Aurelius, was the first to attribute end-organ dysfunction to inflammation [9]. These seemingly simple observations describing localised inflammation still hold true today, and we now have a better understanding of the underlying mechanisms. For example, the swelling and oedema seen in inflammation can be attributed to vasodilation and disruption of the microvascular barrier by inflammatory mediators such as vascular endothelial growth factor (VEGF), causing fenestrations in vascular beds and allowing immune cells such as neutrophils and large molecules including proteins to enter the interstitial space [10]. Net filtration pressure is increased by a combination of a reduction in intravascular colloid osmotic pressure and an increase in capillary pressure and surface area, resulting in oedema.

However, localised inflammation on its own rarely produces the morbidity seen in SIRS. Likewise, bacteraemia is extremely common and may be caused by something as innocuous as toothbrushing, yet sepsis as a consequence of this is relatively rare [11]. What then are the triggers of SIRS in sepsis, if not the mere presence of pathogens within inflamed tissue or the systemic circulation?

10.3 PAMPs and DAMPs: The Molecular Triggers of Inflammation

The inflammatory response is mediated largely by innate immune cells consisting mainly of neutrophils and macrophages. The first step involves the recognition of tissue injury or pathogen invasion via pattern recognition receptors (PRRs) towards damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), respectively [12] (Fig. 10.2). DAMPs are released by host cells and tissue upon injury and include proteins, such as high-mobility group box

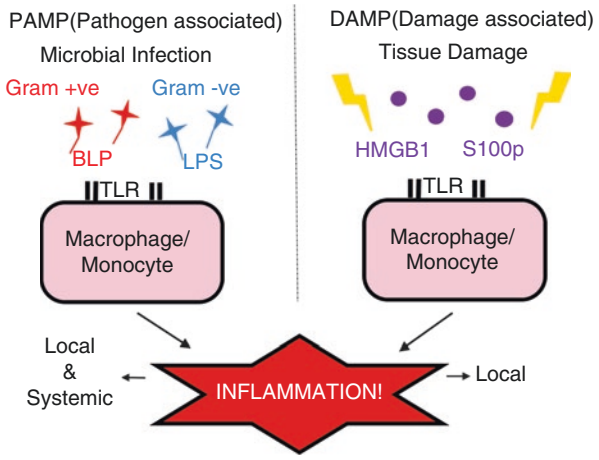

Fig. 10.2 DAMPs and PAMPs are the molecular triggers of inflammation. PAMPs from invading pathogens and DAMPs from tissue damage are recognised by TLR on immune cells, triggering inflammation

(HMGB)-1 and S100 proteins, or RNA, DNA and their nucleotide derivatives such as purine metabolites. In contrast, PAMPs are non-host molecules often derived from the surface of the invading pathogens themselves [13].

The classical example of a PAMP is endotoxin, also known as bacterial lipopolysaccharide (LPS). These are derived from the outer membrane of gram-negative bacteria and usually consist of a glycan polymer termed an O-antigen connected to a lipid A moiety via a core oligosaccharide. LPS can vary greatly between bacteria due to diversity in the O-antigen and core oligosaccharide, but lipid A is highly evolutionarily conserved and is believed to be chiefly responsible for the stimulation of innate immunity resulting in endotoxic shock [14]. Bacterial lipoprotein (BLP) is another potent PAMP and is found on the surface of both gram-positive and gram-negative bacteria. For both, BLP is first synthesised as pre-prolipoprotein before being modified, first by lipoprotein diacyl transferase to prolipoprotein and then by lipoprotein signal peptidase to the mature form of lipoprotein seen in gram-positive bacteria [15]. In gram-negative bacteria, there is further modification by lipoprotein N-acyl transferase prior to maturity. This may explain the different clinical effect of BLP in humans; like LPS, BLP is responsible for much of the virulence of gram-positive bacteria, but this role in gram-negative bacteria is less significant [16].

PAMPs have been evolutionarily selected over millennia to be an effective and reliable signal to trigger our response to pathogen invasion. This is most likely due to their critical functions in bacterial physiology; they are implicated in processes such as adhesion to host cells, the modulation of inflammatory processes and the translocation of virulence factors into host cells [13]. The human immune system has therefore co-evolved to express very specific PRRs towards these antigens. Of these, the Toll-like receptor (TLR) family of proteins are arguably the most extensively studied and best understood, though other PRRs such as RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs) exist as well. Originally named after similar proteins in the *Drosophila* fruit fly where its role in immunity was first

discovered, ten TLRs have so far been described in humans—TLR1, 2, 4, 5, 6 and 10 are expressed on the cell surface, while TLR3, 7, 8 and 9 are endosomal TLRs. They are ubiquitously expressed by multiple cell types throughout the human body and have critical roles not only in pathogen recognition but also in autoimmunity [17]. For the purposes of this chapter, we will focus on their expression by immune cells and their role in the inflammatory response.

10.4 TLR Signalling Is Critical for the Inflammatory Response

TLRs, like the PAMPs they detect, are highly specialised. The TLRs on the cell surface, for example, recognise lipids including LPS and BLP which covers the vast majority of bacteria, while endosomal TLRs recognise nucleic acids such as RNA and DNA thus providing protection against certain viruses. The recognition of PAMPs by TLR may be straightforward via direct binding, but the downstream pathways are complex. They involve adapter molecules whose activity dictates downstream molecular interactions that then go on to modulate the activity of yet further downstream molecules. We will focus on the intracellular pathways triggered following the binding of respective PAMPs to TLR2 and TLR4 here, as they have been shown to be crucial to the immune response towards BLP and LPS, respectively.

The binding of BLP to TLR2 marks the beginning of the adaptor protein myeloid differentiation primary response 88 (MyD88)-dependent pathway which is shared by all TLR receptors including TLR4. To simplify a complex process, members of the interleukin-1 receptor-associated kinase (IRAK) family are recruited following BLP binding to TLR2, which then phosphorylate TNF receptor-associated factor (TRAF)-6. This catalyses the ubiquitination of TRAF-6, transforming growth factor beta-activated kinase (TAK)-1 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) essential modulator (NEMO). This releases IκB kinase (IKK)-α and IKK-β to phosphorylate IκB, which then releases NF-κB to translocate into the nucleus and induce the expression of proinflammatory genes. TAK-1 also has roles in the activation of p38, c-Jun N-terminal kinase (JNK) and activator protein 1 (AP-1)-related responses, which include cellular response to stress, cytokine production, cellular differentiation and apoptosis. TLR3 and TLR4 are unique in that the engagement of TLR3 and TLR4 may result in downstream signalling that is independent of the MyD88 pathway. Instead, this pathway is dependent on TIR-containing adaptor molecule (TICAM)-1, also known as TIR domain-containing adaptor-inducing interferon-β (TRIF). In this alternative pathway, engagement of TLR4 leads to binding of TRIF with thyroid receptor activator molecule (TRAM), which then activates the IKK-related kinases TANK-binding kinase (TBK)-1 and IKK-ε. Interferon regulatory factor (IRF)-3 and IRF-7 are subsequently phosphorylated before translocating into the nucleus, forming homo- and heterodimers, and mediate the transcription of interferon (IFN) and IFN-related genes [17].

10.5 NF-κB Is the Master Regulator of Inflammatory Processes

TLR activation leads to inflammation via NF-κB (Fig. 10.3). NF-κB not only plays a central role in mediating acute inflammation as will be discussed but also mediates chronic inflammation, cancer and autoimmunity. In sepsis, NF-κB induces the expression of cytokines such as interleukin (IL)-1α, IL-1β, IL-6, IL-12, tumour necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which serve to stimulate the production of innate immune cells such as neutrophils [18]. IL-1 and TNF-α in particular are not only produced via NF-κB activation but also feedback into the pathway to stimulate further NF-κB activation in a feed-forward loop, amplifying the inflammatory response [19]. Other NF-κB-induced factors include chemokines such as IL-8, a known chemoattractant for neutrophils, adhesion molecules such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, coagulation factors such as tissue factor and other molecules such as nitric oxide synthase and cyclooxygenase, which together cause a vasodilatory effect with leaky capillaries which could lead to septic shock [20]. Of clinical significance is the finding that inhibition of NF-κB processes results in reduced sepsis-related organ dysfunction, suggesting that careful modulation of this pathway may lead to better outcomes in sepsis.

So far, we have briefly discussed how surgery, trauma and sepsis can lead to the patient morbidity and mortality related to a dysregulated host inflammatory response. In most cases, this is initiated by PAMPs binding PRRs, followed by the activation of downstream molecules and the expression of proinflammatory genes. The resultant release of proinflammatory cytokines and chemokines produces the clinical picture seen. We will next detail the concept of immune tolerance and discuss its potential in sepsis.

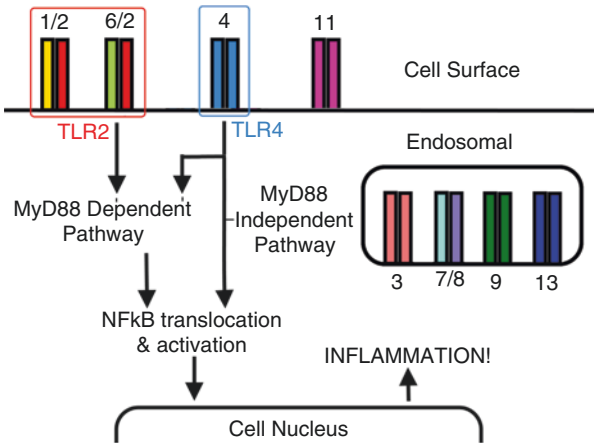

Fig. 10.3 TLR activation leads to inflammation via NF-κB. Cell surface TLRs 1, 2 and 6 form heterodimers and 4 and 11 form homodimers. Endosomal TLRs 3, 9 and 13 form homodimers and 7 and 8 form heterodimers. TLR2 and TLR4 signalling briefly illustrated here, leading to NF-κB activation and inflammation either dependent or independent of the MyD88 pathway

10.6 A Brief History of Tolerance

Fever, a term for an abnormal rise in body temperature above the physiologic norm, may be the best known sign of systemic inflammation. In the first half of the twentieth century, the typhoid vaccine, available then only as a suspension of killed bacteria, was employed not only to prevent typhoid fever but also to treat it [21]. Known as fever therapy, the vaccine was injected intramuscularly in patients to induce a fever in the belief that the Vi antibody within could suppress the multiplication of bacteria, while the O-antigen, the self-same one found in bacterial LPS, could neutralise bacterial endotoxins [22]. Clinicians of the day noted that ever-increasing quantities of vaccine were required to produce the same effect on body temperature. This resistance to the vaccine was attributable to just the pyrogenic fraction of the vaccine, endotoxin, and is now known as "tolerance".

This clinical observation was demonstrated in a rabbit experimental model by Paul Beeson in 1947, when he showed that repeated injection of the typhoid vaccine caused a progressive reduction in vaccine-induced fever in rabbits. Additionally, he showed that this tolerance had a finite duration with diminishing effect over time, as rabbits given the vaccine in weekly intervals exhibited reduced fever suppression, while rabbits allowed to rest for 3 weeks lost their tolerance entirely [23]. Watson and Kim went on to show that rabbits that developed tolerance were resistant to a dose of endotoxin that would have been lethal to non-tolerant animals, demonstrating the potential clinical benefit of this observation [24].

However, the mechanisms behind tolerance proved difficult to elucidate. That bacterial endotoxin was key was shown early on, when tolerance to endotoxin derived from enteric bacilli was observed in patients convalescent from typhoid and parathyroid fevers [25]. Interestingly, this work also showed the non-specificity of the endotoxin required for the development of tolerance. Freedman showed that tolerance may be conferred to a non-tolerant animal by passive transfer of serum [26]. Together, these findings suggested an underlying mechanism that was dependent on activating factors within the blood rather than on inhibitory factors such as antibodies. It was postulated that cells of the reticuloendothelial system were implicated, as blockage with colloid nullified tolerance in rabbits. Freudenberg and Galanos confirmed this hypothesis much later when they showed that transfer of monocytes/macrophages from LPS-tolerant mice conferred said tolerance to LPS-naïve mice [27].

10.7 The Role of TLRs in Tolerance

We have established that exposure to LPS results in a dampening of the inflammatory response to subsequent LPS exposure and that this effect is mediated at least in part by monocytes/macrophages. However, the mechanisms involved are not fully elucidated and remain one of the greatest challenges in the field today. Endotoxin tolerance may be regulated at any number of sites, and the binding of endotoxin to TLR4 on monocytes/macrophages seems to be the first step in the majority of cases.

IL-1 was found to be the cytokine responsible for fever by Paul Beeson in the 1940s and, together with TNF-α, is also one of the major cytokines responsible for the inflammatory response. As such, reduced IL-1 and TNF-α production have been used as a measure of tolerance in studies. Interestingly, IL-1, but not TNF-α, was shown to also be able to induce tolerance to endotoxin shock, with a concomitant downregulation of TLR4, suggesting that IL-1-induced tolerance stems from reduced recognition of LPS in tolerant cells [28]. This finding was supported by Nomura et al. who showed in a mouse model that peritoneal macrophages pre-exposed to LPS and thus tolerised were found to have both a reduction in TLR4 messenger-RNA (mRNA) expression and surface TLR4 expression within hours of the tolerising event. While the former returned to original levels within 24 h, the suppression of the latter persisted, illustrating the memory associated with tolerance. Upon re-exposure to LPS, the activation of neither IRAK nor NF-κB was observed in these tolerised macrophages [29]. It seemed reasonable to conclude that the lack of LPS-TLR4 binding and subsequently the non-activation of the TLR4-MyD88-dependent pathway were at least partly responsible for the phenomenon of tolerance in these cells.

Compared to LPS-induced tolerance, BLP-induced tolerance is relatively newly described. Nonetheless, they share many similarities and differences which will be discussed throughout this chapter. Wang et al. were the first to demonstrate BLP tolerance by showing that pretreatment of human THP-1 monocytes with synthetic BLP resulted in diminished TNF-α and IL-6 production when treated with a second BLP challenge, ergo, a reduced inflammatory response. BLP-tolerised cells also displayed reduced TLR2 surface expression compared to BLP naïve cells upon re-exposure to BLP, paralleling the findings seen with TLR4 in LPS tolerance. Furthermore, the degree of suppression of mitogen-activated protein (MAP) kinase phosphorylation and NF-êB activation in BLP tolerance was found to be similar to that seen in LPS-induced tolerance [30]. Together, these results reinforce the importance of TLR signalling in the induction of tolerance and suggest that LPS and BLP tolerance may share intracellular pathways despite having different triggers, just as TLR4 and TLR2, respectively, both activate the same NF-κB pathway.

10.8 Cross-Tolerance Between BLP and LPS

Given the similarities between LPS and BLP tolerisation in terms of method of activation and molecular pathways involved, one could hypothesise that a tolerising event by one would affect the response of the cell or organism to the other. Wang et al. demonstrated “cross-tolerance” between the two, showing that TNF-α and IL-6 production was reduced in BLP-tolerised cells when restimulated with either BLP or LPS. Surprisingly, this result was not seen in LPS-tolerised cells in response to BLP. Additionally, CD14, a membrane-bound protein that has a critical role in the recognition and binding of LPS, was found to be inconsequential to the induction of BLP tolerance or cross-tolerance to LPS [30]. This finding is significant for two reasons. Firstly, if BLP tolerance results in tolerance to LPS as well,

independent of LPS/CD14/TLR4 signalling, then the mechanism of tolerance is downstream of these surface molecules, and their effect is only partial at best. Secondly, this emphasises the differences between BLP and LPS tolerance that despite their similarities, they have different intracellular effects which, when combined, may dictate different outcomes for the organism in question.

Indeed, while both LPS and BLP tolerance seem to produce similar effects on cytokine production in vitro, other factors come into play in in vivo models of tolerance. When BLP-tolerised, LPS-tolerised or non-tolerised mice were subjected to BLP or a combined BLP/LPS challenge, the BLP-tolerised mice were surprisingly resilient with no mortality seen at 72 h after injection compared to the others with mortality rates ranging from 60 to 80%. When BLP-tolerised mice were challenged with LPS, 60% were alive at 72 h, compared to only 20% for non-tolerised mice. Of potential clinical significance, when both BLP- and LPS-tolerised mice were subjected to bacterial sepsis either via injection of *S. aureus* and *S. typhimurium* or from caecal ligation and puncture (CLP)-induced polymicrobial sepsis, the BLP-tolerised cohort consistently had better outcomes, with survival rates of 40–60% compared to 0–30% seen in the other mice [31]. While LPS tolerance was shown to confer a survival advantage against LPS-induced lethality, BLP tolerance seemed to confer protection against both BLP- and LPS-induced lethality. What could explain this difference, given that both BLP and LPS activate the NF-κB pathway via TLRs as previously described?

The superiority of BLP tolerance compared to LPS tolerance, especially in the in vivo models of sepsis described, may be a simple reflection of bacterial antigen expression, as both gram-positive and gram-negative bacteria express BLP, while LPS is only expressed by gram-negative bacteria. However, BLP tolerance has been demonstrated to have additional antimicrobial effects beyond the suppression of proinflammatory cytokines seen in tolerance so far. Indeed, BLP tolerance results in an overexpression of complement receptor type 3 (CR3) and FcγIII/II receptor (FcγIII/IIR) on both polymorphonuclear neutrophils (PMNs) and peritoneal macrophages, which may increase their bacterial recognition and bactericidal activity [31]. O'Brien et al. used TLR4-deficient C3H/HeJ mice which are highly susceptible to gram-negative sepsis to further study the effect of BLP tolerance on bacterial clearance [32]. As before, BLP tolerance conferred a survival advantage at 72 h after injection with live *S. typhimurium*, a gram-negative bacteria, with significantly concomitant increases in CR3 and FcγIII/IIR expression on PMNs. PMNs and macrophages from the BLP-tolerised C3H/HeJ mice displayed significantly increased uptake, ingestion and intracellular killing of *S. typhimurium*, accompanied by an upregulation of inducible nitric oxide (NO) synthase and increased production of intracellular NO by peritoneal macrophages from BLP-tolerised mice [32].

Clearly, there are mounting evidence of the clinical utility of BLP tolerance and its cross-tolerance to LPS. If TLR signalling is of secondary importance as demonstrated by cross-tolerance, then the key must lie within the intracellular signalling pathways stimulated by BLP and LPS recognition, and the NF-κB pathway seems to be central to this.

10.9 Signalling Upstream of NF-κB

While TLR binding seems to be the common initiating step for the induction of tolerance, it is by no means required. The modulation of signalling molecules downstream of TLR binding, or alternative activation of the NF-κB pathway such as by IL-1 signalling mentioned earlier, may be sufficient to cause tolerisation. The importance of the MyD88-dependent pathway to NF-κB activation is exemplified when Kawai et al. found that MyD88-deficient mice were not only unresponsive to endotoxin but also became resistant to LPS-induced shock [33]. In addition, LPS-tolerised monocytes displayed inhibited IRAK expression, kinase activity and association with MyD88, consequently resulting in reduced NF-κB activation and reduced expression of proinflammatory cytokines [28].

Similar to LPS tolerance, BLP-tolerised THP-1 monocytes had markedly reduced IRAK-1 protein expression and MyD88-IRAK immunocomplex formation, as demonstrated by immunoprecipitation in BLP-tolerised cells following a second BLP or LPS challenge [34]. This resulted in reduced TNF-α production in response to both BLP and LPS in these cells. Li et al. additionally show that IRAK-1 overexpression negates BLP-induced tolerance [34]. This may be through the modulation of p65 phosphorylation and binding to TNF-α and IL-6 gene promoters, as BLP-tolerised cells exhibit suppressed nuclear transactivation of p65 at these sites which was restored with IRAK-1 overexpression in an in vitro model [34]. Despite this, IRAK-1 mRNA expression remained unchanged, as were the mRNA expression of TLR2, TLR4, MyD88, IRAK-4 and TRAF6 in BLP-tolerant THP-1 cells [34]. This may be due to post-transcriptional modulation that will be discussed later in this chapter.

Medvedev et al. corroborated these findings, while also showing that extracellular signal-regulated kinases, c-Jun NH2-terminal kinases and p38 kinase activity was inhibited in tolerant macrophages, suggesting that there may be multiple levels to the regulation of endotoxin tolerance within the MyD88-dependent pathway, ultimately resulting in the reduced expression of proinflammatory cytokines such as GM-CSF, IFN-γ-inducible protein-10, KC, JE/monocyte chemoattractant protein-1, macrophage-inflammatory protein-1β and macrophage-inflammatory protein-2 [35]. However, other accessory proteins affect this pathway too, an example of which is the ST2 protein. The orphan receptor ST2, also known as T1 and DER4, is a member of the TIR domain-containing superfamily [36]. ST2 negatively regulates TLR4 signalling by inhibiting IκB-α degradation and also via interference with the MyD88 and Mal adaptor proteins, which results in a reduction in LPS-stimulated proinflammatory cytokine production [37]. Crucially, in the context of tolerance, ST2 functions as a mediator for inducing LPS tolerance as both ST2-deficient macrophages and mice failed to develop LPS tolerance [38]. These findings were mirrored to a certain extent in BLP tolerance, as ST2-deficient macrophages demonstrated significantly enhanced MyD88-IRAK and MyD88-TLR2 immunocomplex formation in response to BLP stimulation, leading to increased production of proinflammatory cytokines [39]. In contrast, overexpression of ST2 inhibited BLP-induced NF-κB activation, adding evidence to the

negative regulatory role of ST2 in the inflammatory response. However, unlike in LPS tolerance, the production of TNF-α and IL-6 was attenuated in BLP-tolerised but ST2-deficient macrophages in response to a second BLP stimulation, suggesting that BLP tolerance develops even in the absence of the ST2 receptor. This finding is further supported by in vivo models showing improved survival in ST2-deficient but BLP-tolerised mice when further challenged with an otherwise lethal dose of BLP following tolerisation [39].

The evidence so far points to the NF-κB pathway being the primary pathway involved in the induction of tolerance and its effects. Most of the effects of tolerised cells result in an inhibition of this pathway leading to reduction in pro-inflammatory cytokine production and consequently the systemic effects of the inflammatory response. However, the production of these cytokines is but one facet of the myriad functions of the NF-κB pathway. Indeed, in studying the mechanism of bacterial clearance seen in BLP tolerance, Liu et al. found that BLP-tolerised monocytes/macrophages display increased bactericidal properties that were dependent on NF-κB pathway activation. These cells surprisingly displayed increased NF-κB activation following restimulation with *S. aureus* or *S. typhimurium* and confirmed by imaging of increased nuclear translocation of p65. Despite this finding, the expected effect of tolerisation on upstream pathways of NF-κB including TLR2, MyD88 and IRAK-1 expression was seen. This unique observation was accompanied by accelerated phagosome maturation and upregulated expression of membrane-trafficking regulators and lysosomal enzymes that may be responsible for the enhanced antibacterial activity seen in BLP-tolerised monocytes/macrophages [40].

10.10 Post-transcriptional Modulation of NF-κB

A recurring observation seen in tolerance is reduced protein expression in tolerised cells despite normal or near-normal mRNA expression. This discrepancy may be explained by the effect of microRNAs (miRNAs), some of which are upregulated in tolerance. miRNAs are a class of evolutionarily conserved short RNA sequences that work to downregulate protein expression by binding to the 3′ untranslated (UTR) region of mRNA, resulting in either their degradation of inhibiting their translation [41, 42]. Since their discovery, several miRNAs have been shown to be upregulated in response to LPS stimulation. IRAK-1 and TRAF-6 are key molecules in the NF-κB pathway and found to be targets of miR-146a, which was shown to be upregulated following repeat LPS challenge. Inflammatory responses towards not only TLR4 but also TLR2 ligands were reduced accordingly [43]. In LPS-tolerised THP-1 monocytes, TNF-α production was diminished following re-exposure to LPS, and this was correlated with an increase in miR-146a expression in a dose-dependent manner. Interestingly, the upregulation of miR-146a alone mimics LPS priming to induce LPS tolerance, and miR-146a knockdown similarly reduced LPS tolerance, showing that the pre-transcriptional steps of the pathway may potentially be redundant [44]. This is significant as it suggests that miR-146a

may be a key player in LPS tolerance by influencing gene expression at the post-transcriptional level. Since miR-146a affects TLR2 ligand-induced inflammatory responses as well, it was speculated that miR-146a may have effects in BLP tolerance as well. Indeed, just as with LPS tolerance, miR-146a was upregulated by BLP restimulation in BLP-tolerised THP-1 monocytes and accompanied by reductions in IRAK-1 and phosphorylated IkB-α expressions. More importantly, this effect persisted when BLP-tolerised cells were treated with heat-killed gram-negative *S. typhimurium* [45]. This provides further evidence of BLP and LPS cross tolerisation, where tolerance to BLP is accompanied by tolerance to LPS, with significant clinical implications.

10.11 DAMP-PAMP Interactions in Tolerance

PAMPs such as LPS and BLP have been the subject of discussion so far as they are not only both key to the effect of tolerance but also primarily responsible for the dysregulated inflammatory response leading to toxicity and death. However, it is important to recognise and appreciate the input from other signalling molecules derived from non-pathogenic sources. DAMPs are often acute phase molecules secreted by immune cells that may serve to sustain or even escalate an inflammatory response triggered by PAMP recognition.

Myeloid-related protein 8 (Mrp8), also known as S100A8, is part of a family of proteins called the S100 proteins and forms the active component of the Mrp8/14 protein complex [46]. It functions as a molecular DAMP, being released by phagocytes at the site of infection, and supports the inflammatory response by being involved in the mechanism of recruitment of inflammatory cells to the site of injury [47]. Extracellularly, Mrp8/14 serves other functions including anti-proliferative, antitumoural, anti-nociceptive and, in the context of inflammation and sepsis, antibacterial activities [48]. As a DAMP, Mrp8/14 also binds to and activates TLR4 and has been shown to amplify LPS-induced TNF-α release by inducing the translocation of MyD88 and activating IRAK-1, thus activating the NF-κB pathway. This makes Mrp8/14 a potent factor leading to LPS-induced septic shock and lethality, demonstrated by the observation that Mrp14 gene knockout mice were protected against LPS-induced lethal shock [49]. The overlap between Mrp8 and 14 with tolerance-related cellular processes suggests that it may be involved in the latter. Coveney et al. demonstrated that similar to LPS and BLP, monocytes/macrophages may be induced to grow tolerant to Mrp8/14 restimulation after an initial "priming" event. However, the effect of tolerance seen by Mrp8/14-tolerised cells was evidenced not only to subsequent Mrp8/14 restimulation but also to both LPS and BLP, potentially highlighting this pathway in cross-tolerance. Like LPS tolerance, Mrp8/14-tolerised cells exhibited substantially attenuated TNF-α and IL-6 release both locally and in the systemic circulation and, as in BLP tolerance, increased PMN recruitment and accelerated bacterial clearance [50]. Interestingly, both TLR4 and TLR2 seem to contribute to Mrp8/14 tolerance, suggesting that Mrp8/14 tolerance may be common to both BLP and LPS tolerance.

Another example involves high-mobility group box (HMGB)-1 protein, which serves as a nuclear protein with roles in the regulation of DNA transcription [51], and also functions as a DAMP when secreted by activated monocytes/macrophages. HMGB-1 binds LPS to form an immunostimulatory complex which results in increased NF-κB activation and IL-6 production. Interestingly, exposure of HMGB-1 to LPS increases the expression of HMGB-1, resulting in a feed-forward loop amplifying the inflammatory response leading to SIRS [52]. Unsurprisingly, inhibition of HMGB-1 has been shown to lead to improved survival in mice with established lethal sepsis and systemic inflammation [53]. In contrast, BLP tolerisation leads to a reduction in HMGB-1 gene expression and protein expression in an in vitro model using THP-1 monocytic cells [54]. In vivo, BLP tolerisation was associated with not only an attenuation in serum HMGB-1 levels but also of HMGB-1 levels in peritoneal macrophages, leading to improved survival of C57BL/6 mice challenged with a lethal dose of BLP. Perhaps most significantly from a clinical perspective, the neutralisation of HMGB-1 using anti-HMGB-1 antibodies abrogated BLP-associated lethality almost completely [54]. Together, these findings suggest a pathway to limiting SIRS via BLP tolerisation that is not seen in LPS tolerisation and at least partially explains the superior clinical effect of BLP tolerance compared to LPS tolerance.

10.12 Clinical Applications of Tolerance

The potential clinical applications of tolerance are vast, but this area is very much in its infancy. As both BLP and LPS tolerance reduces inflammation from subsequent infection, the use of BLP and/or LPS as vaccines has been considered, but their use in clinical practice is severely limited owing to their toxicity resulting in severe SIRS and consequently death. Instead, research has been focused on the pathways involved in tolerance, the molecular mechanisms therein and how we may potentially modulate them to produce the effect of tolerance without the need for either BLP or LPS challenge, thus avoiding their lethal effects.

Despite their tremendous toxicity, the idea of BLP or LPS as an antibacterial vaccine should not be dismissed. Edward Jenner developed the first vaccine by inoculating people with cowpox to prevent smallpox in 1796. This caused cowpox, which was a relatively mild disease especially when compared to smallpox which was often deadly. Thanks to his insight, innovation and vision, he was able to see beyond the immediate short-term complications of cowpox, and less than 200 years later in 1977, smallpox was completely eradicated from this world, with not a single new case since. There now exists a myriad of vaccines against multiple viral pathogens, many of which used to cause high morbidity and mortality, but which are now also on their way to extinction due to vaccination. Many of these vaccines have been modified or were synthesised to result in protection from their respective pathogens while minimising their harmful effects. Drawing parallels, there is enormous potential for bacterial vaccination with tolerance. More work needs to be done to produce

an LPS or BLP analogue which would result in tolerance without triggering SIRS, and cellular reprogramming may be key to this.

Instead of bacterial vaccination, it may also be possible to improve outcomes in sepsis via tolerance by indirect means. We mentioned cellular reprogramming earlier, but research on tolerance has revealed multiple other cellular processes as described in this chapter that all contribute towards the effect and play a role in sepsis overall. Careful modulation of these targets may result in improved outcomes. With the rise of antimicrobial resistance and the emergence of multidrug-resistant organisms, more preventative and/or treatment modalities are sorely required in the global fight against sepsis, and tolerance could prove to be the answer. Also, most of the discussion in this chapter has been with regard to the role of tolerance in inflammation related to sepsis, yet we know from work in other but related fields that inflammation is core to other disease processes, both those traditionally known to be inflammatory such as inflammatory bowel disease, vasculitis or the inflammatory arthropathies and also perhaps less obvious but nonetheless clinically relevant diseases such as atherosclerosis, diabetes and cancer. Further insight into the workings of tolerance may shed light on these other related diseases and pave the way for new, exciting and effective therapies.

10.13 Summary

In summary, we have in this chapter discussed how trauma, surgery and sepsis are related and how they account for a substantial proportion of global disease burden, with sepsis in particular having a high morbidity and mortality. Inflammation plays a key role in sepsis, not only as a mechanism to combat invading foreign pathogens but also via its detrimental effects on the host in cases of inflammatory overstimulation. The triggers of the molecular mechanisms relevant to inflammation in sepsis include PAMPs and DAMPs, which in turn activate TLR signalling, and, ultimately, the NF-κB pathway, which then regulates other downstream inflammatory processes.

Tolerance as a concept is relatively new, with its effects being described just in the beginning of the last century and its molecular mechanisms only being studied closely in the last 20 years. Tolerance induced by BLP or LPS exposure, through some or all of the previously discussed molecules and pathways, result in a dampening of the systemic inflammatory response in response to subsequent exposure of BLP or LPS, potentially reducing their lethality. Given the many similarities between LPS and BLP tolerance, the phenomenon of cross-tolerance was discussed, in which we highlighted several key differences between the two. BLP tolerance, while being relatively less studied, may have a superior clinical outcome when compared to LPS tolerance. BLP demonstrates cross-tolerance to LPS but not vice versa, and only BLP tolerance so far has resulted in a survival benefit from bacterial sepsis in in vivo models. Despite the paucity of clinical studies in this field, the potential clinical applications from the study of tolerance are evident. Clearly, further vigorous study into this interesting phenomenon should be done and efforts made to bring the results from the bench to the bedside.

References

1. Singer M, Deutschman CS, Seymour C, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA. 2016;315(8):801–10. https://doi.org/10.1001/jama.2016.0287.
2. Lagu T, Rothberg MB, Shieh M-S, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. Crit Care Med. 2012;40(3):754–61. https://doi.org/10.1097/CCM.0b013e318232db65.
3. Mann EA, Baun MM, Meininger JC, Wade CE. Comparison of mortality associated with sepsis in the burn, trauma, and general intensive care unit patient: a systematic review of the literature. Shock. 2012;37(1):4–16. https://doi.org/10.1097/SHK.0b013e318237d6bf.
4. Ou L, Chen J, Burrell T, et al. Incidence and mortality of postoperative sepsis in New South Wales, Australia, 2002–2009. Crit Care Resusc. 2016;18(1):9–16.
5. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996;22(7):707–10.
6. Marshall JC. Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome. Crit Care Med. 2001;29(7 Suppl):S99–106.
7. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med. 2003;29(4):530–8. https://doi.org/10.1007/s00134-003-1662-x.
8. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644–55.
9. Granger D, Senchenkova E. Historical perspectives. In: Inflammation and the microcirculation. San Rafael: Morgan & Claypool Life Sciences; 2010.. https://www.ncbi.nlm.nih.gov/books/NBK53379/.
10. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci*. 1995;108(6):2369–79.. http://jcs.biologists.org/content/108/6/2369.abstract
11. Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. Bacteremia associated with tooth brushing and dental extraction. Circulation. 2008;117(24):3118–25. https://doi.org/10.1161/CIRCULATIONAHA.107.758524.
12. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140(6):805–20. https://doi.org/10.1016/j.cell.2010.01.022.
13. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol Rev. 2012;249(1):158–75. https://doi.org/10.1111/j.1600-065X.2012.01146.x.
14. Rietschel ET, Kirikae T, Schade FU, et al. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J. 1994;8(2):217–25.. http://www.fasebj.org/content/8/2/217.abstract
15. Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 2001;7(3):167–202.
16. Kovacs-Simon A, Titball RW, Michell SL. Lipoproteins of bacterial pathogens. Infect Immun. 2011;79(2):548–61. https://doi.org/10.1128/IAI.00682-10.
17. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4(7):499–511. https://doi.org/10.1038/nri1391.
18. Lawrence T. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1(6):a001651. https://doi.org/10.1101/cshperspect.a001651.
19. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87–103.. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3066460/

20. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets—an updated view. Mediat Inflamm. 2013;2013:165974. https://doi.org/10.1155/2013/165974.
21. Climie H. Immunization against typhoid and paratyphoid with alcohol-killed, alcohol-preserved and heat-killed, phenol-preserved, vaccine. J Hyg (Lond). 1942;42(4):411–5.
22. Hodgson AE. Specific serum therapy in typhoid. Br Med J. 1944;2(4366):339–40.. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2286195/
23. Beeson PB. Tolerance to bacterial pyrogens: I. Factors influencing its development. J Exp Med. 1947;86(1):29–38.
24. Watson DW, Kim YB. Modification of host responses to bacterial endotoxins. I. specificity of pyrogenic tolerance and the role of hypersensitivity in pyrogenicity, lethality, and skin reactivity. J Exp Med. 1963;118:425–46.
25. Neva FA, Morgan HR. Tolerance to the action of endotoxins of enteric bacilli in patients convalescent from typhoid and paratyphoid fevers. J Lab Clin Med. 1950;35(6):911–22.
26. Freedman HH. Passive transfer of tolerance to pyrogenicity of bacterial endotoxin. J Exp Med. 1960;111(4):453–63.. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2137276/
27. Freudenberg MA, Galanos C. Induction of tolerance to lipopolysaccharide (LPS)-D-galactosamine lethality by pretreatment with LPS is mediated by macrophages. Infect Immun. 1988;56(5):1352–7.
28. Dinarello CA. The history of fever, leukocytic pyrogen and interleukin-1. Temp Multidiscip Biomed J. 2015;2(1):8–16. https://doi.org/10.1080/23328940.2015.1017086.
29. Nomura F, Akashi S, Sakao Y, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. J Immunol. 2000;164(7):3476–9.
30. Wang JH, Doyle M, Manning BJ, di Wu Q, Blankson S, Redmond HP. Induction of bacterial lipoprotein tolerance is associated with suppression of toll-like receptor 2 expression. J Biol Chem. 2002;277(39):36068–75. https://doi.org/10.1074/jbc.M205584200.
31. Wang JH, Doyle M, Manning BJ, et al. Cutting edge: bacterial lipoprotein induces endotoxin-independent tolerance to septic shock. J Immunol. 2003;170(1):14–8.
32. O'Brien GC, Wang JH, Redmond HP. Bacterial lipoprotein induces resistance to gram-negative sepsis in TLR4-deficient mice via enhanced bacterial clearance. J Immunol. 2005;174(2):1020–6.
33. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity*. 1999;11(1):115–22. https://doi.org/10.1016/S1074-7613(00)80086-2.
34. Li CH, Wang JH, Redmond HP. Bacterial lipoprotein-induced self-tolerance and cross-tolerance to LPS are associated with reduced IRAK-1 expression and MyD88-IRAK complex formation. J Leukoc Biol. 2006;79(4):867–75. https://doi.org/10.1189/jlb.0905505.
35. Medvedev AE, Kopydlowski KM, Vogel SN. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J Immunol. 2000;164(11):5564–74.
36. Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. FEBS Lett. 1989;258(2):301–4.
37. Sweet MJ, Leung BP, Kang D, et al. A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of toll-like receptor 4 expression. J Immunol. 2001;166(11):6633–9.
38. Brint EK, Xu D, Liu H, et al. ST2 is an inhibitor of interleukin 1 receptor and toll-like receptor 4 signaling and maintains endotoxin tolerance. Nat Immunol. 2004;5(4):373–9. https://doi.org/10.1038/ni1050.
39. Liu J, Buckley JM, Redmond HP, Wang JH. ST2 negatively regulates TLR2 signaling, but is not required for bacterial lipoprotein-induced tolerance. J Immunol. 2010;184(10):5802–8. https://doi.org/10.4049/jimmunol.0904127.
40. Liu J, Xiang J, Li X, et al. NF-κB activation is critical for bacterial lipoprotein tolerance-enhanced bactericidal activity in macrophages during microbial infection. Sci Rep. 2017;7:40418. https://doi.org/10.1038/srep40418.

41. Martinez J, Tuschl T. RISC is a 5′ phosphomonoester-producing RNA endonuclease. Genes Dev. 2004;18(9):975–80. https://doi.org/10.1101/gad.1187904.
42. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. Science. 2002;297(5589):2056–60. https://doi.org/10.1126/science.1073827.
43. Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A. 2006;103(33):12481–6. https://doi.org/10.1073/pnas.0605298103.
44. Nahid MA, Pauley KM, Satoh M, Chan EKL. miR-146a is critical for endotoxin-induced tolerance: implication in innate immunity. J Biol Chem. 2009;284(50):34590–9. https://doi.org/10.1074/jbc.M109.056317.
45. Quinn EM, Wang JH, O'Callaghan G, Redmond HP. MicroRNA-146a is upregulated by and negatively regulates TLR2 signaling. PLoS One. 2013;8(4):e62232. https://doi.org/10.1371/journal.pone.0062232.
46. Roth J, Vogl T, Sorg C, Sunderkotter C. Phagocyte-specific S100 proteins: a novel group of proinflammatory molecules. Trends Immunol. 2003;24(4):155–8.
47. Rammes A, Roth J, Goebeler M, Klempt M, Hartmann M, Sorg C. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. J Biol Chem. 1997;272(14):9496–502.
48. Steinbakk M, Naess-Andresen C-F, Fagerhol MK, Lingaas E, Dale I, Brandtzaeg P. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. *Lancet*. 1990;336(8718):763–5. https://doi.org/10.1016/0140-6736(90)93237-J.
49. Vogl T, Tenbrock K, Ludwig S, et al. Mrp8 and Mrp14 are endogenous activators of toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med. 2007;13(9):1042–9. https://doi.org/10.1038/nm1638.
50. Coveney AP, Wang W, Kelly J, et al. Myeloid-related protein 8 induces self-tolerance and cross-tolerance to bacterial infection via TLR4- and TLR2-mediated signal pathways. Sci Rep. 2015;5:13694. https://doi.org/10.1038/srep13694.
51. Klune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB1: endogenous danger signaling. Mol Med. 2008;14(7–8):476–84. https://doi.org/10.2119/2008-00034.Klune.
52. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science*. 1999;285(5425):248–51.. http://science.sciencemag.org/content/285/5425/248.abstract
53. Ulloa L, Ochani M, Yang H, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc Natl Acad Sci U S A. 2002;99(19):12351–6. https://doi.org/10.1073/pnas.192222999.
54. Coffey JC, Wang JH, Kelly R, et al. Tolerization with BLP down-regulates HMGB1 a critical mediator of sepsis-related lethality. J Leukoc Biol. 2007;82(4):906–14. https://doi.org/10.1189/jlb.0806504.

New Progress of Goal-Directed Fluid Resuscitation for Septic Shock

11

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Abstract

Fluid resuscitation is the cornerstone of resuscitation in patients with septic shock. In 2001, Rivers presented the early goal-directed therapy (EGDT) strategies to direct intravenous infusion for septic shock patients. As a result, EGDT was adopted by the Surviving Sepsis Campaign. Recently a trio of trials (ProCESS, ARISE, and ProMISe) question the continued need for all of the elements of EGDT or the need for protocolized care for patients with septic shock. Nevertheless, it is important to find the right goal for septic shock to direct fluid resuscitation. However, it must be recognized that due to the pathophysiological characteristics of septic shock, the goals of resuscitation at different stages of shock are different. Therefore, in the continuous process of the development of septic shock, we must fully understand the meaning and limitations of various parameters, so as to select the right goal to direct the fluid resuscitation therapy. In this chapter, we will introduce all kinds of parameters used to guide fluid resuscitation for septic shock patients. And some new parameters including metabolic parameters and dynamic parameters will be highlighted in this chapter.

Keywords

Fluid resuscitation · Septic shock · Goal-directed fluid resuscitation (EGDT) · Fluid challenge · Lactate (LAC) and lactate clearance · Stroke volume variation (SVV) · Pulse pressure variation (PPV) · End-expiratory occlusion test (EEOt) · Superior/inferior vena cava collapsibility index (SVC-CIIVC-CI) · Tidal volume challenge

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11.1 Overview

According to the definition of Sepsis-3, septic shock is defined as a subset of sepsis in which underlying circulator and cellular metabolism abnormalities are profound enough to substantially increase mortality. Adult patients with septic shock can be identified using the clinical criteria of a vasopressor requirement to maintain a mean arterial pressure (MAP) greater than or equal to 65 mmHg and a lactate measure greater than 2 mmol/L after adequate volume resuscitation [1]. According to its hemodynamic characteristics, septic shock is a type of distributive shock. The relative hypovolemia is the basic pathophysiological changes of septic shock. The hypovolemia exacerbates insufficient tissue perfusion of systemic organs, thus the oxygen supply cannot meet the metabolic needs, and then lead to multiple organ dysfunction.

Fluid resuscitation is an important part of management of septic shock; it compensates the circulating blood volume at early stage to quickly correct the tissue perfusion problems. Fluid resuscitation therapy should be performed at early stage of septic shock, and the repeated assessments and adjustment are needed in the whole therapeutic process, until the hemodynamic stability is achieved. Fluid resuscitation is not an isolated treatment for septic shock; clinicians are required to continually assess and intervene the cardiac function and vascular tone accordingly.

11.2 Goal-Directed Fluid Resuscitation

Early and effective fluid resuscitation is crucial for the treatment of septic shock. Fluid resuscitation should begin immediately after the diagnosis of hypovolemia and shock. Volume status refers to the cardiac preload; hypovolemia refers to the effective blood volume which is lower than normal. Absolute or relative hypovolemia is a common cause of acute circulatory failure or insufficient tissue perfusion. Fluid resuscitation is an important means of restoring effective blood volume, improving circulatory status and tissue perfusion. However, critically ill patients are often complicated with increased vascular permeability and organ dysfunction. New researches show that nearly half of critically ill patients in ICU have no fluid responsiveness due to various reasons. For these patients, aggressive fluid resuscitation may not improve hemodynamics status and tissue perfusion, but it may also aggravate tissue edema and even induce the pulmonary edema and respiratory failure. Thus, hemodynamic monitoring of patients with circulatory failure not only helps to understand the causes of circulatory failure but also can identify specific treatment goals through hemodynamic parameters and then identify the need for fluid resuscitation and other managements and further adjustment of the fluid resuscitation treatment according to the resuscitation response [2]. It is very important to use hemodynamic parameters to direct the early fluid resuscitation for septic shock. The best treatment to maintain hemodynamic stable for patients with septic shock is early resuscitation. The initial goal for fluid resuscitation is to ensure adequate tissue perfusion; the ultimate goal is to rebuild oxygen supply/demand balance of the

tissues and restore organ function. The assessment of the endpoint of resuscitation includes the clinical parameters, hemodynamic parameters, and oxygen metabolic parameters. Hemodynamic parameters consist of pressure parameters, volume parameters, and blood flow parameters. The above parameters can be further classified into static and dynamic parameters [3]. Each static and dynamic parameter has its own special meaning, which needs to be analyzed properly to achieve goal-directed fluid resuscitation for septic shock.

11.3 Clinical Parameters

Common clinical parameters include urine output, consciousness, and signs of peripheral perfusion such as mottling score as well as capillary refill time [4, 5].

11.3.1 Urine Output

Urine output can reflect the renal perfusion; it is an important parameter for the evaluation of acute renal injury in sepsis [6]. For patients with septic shock, the goal of maintaining a patient's urine output >0.5 mL/kg/h is necessary, but monitoring of urine output often lags behind other functional parameters such as collapse index of the inferior vena cava [7]. And targeting oliguria reversal in goal-directed hemodynamic management does not reduce renal dysfunction in perioperative and critically ill patients [8]. In addition, monitoring of the urine output is not accurate. The new monitoring instrument—electronic urinary output monitoring with self-clearing drainage—may help clinicians to monitor urine output more accurately [9].

11.3.2 Mottled Skin

Patients with septic shock develop the mottled skin which is characterized by uneven mottling of the skin, reflecting the peripheral perfusion of the skin. The mottling is usually more obvious at the knees. Motting score ranges 0 to 5, the higher the score indicates the greater area of mottled skin, such as mottling limited to the knees and the mottling of the entire legs. The higher the mottling score predicts the worse prognosis in patients with septic shock [10].

11.3.3 Capillary Refill Time

Capillary refill time (CRT) can be measured by press the fingers, especially the fingertip of the index finger, until it turns white, then taking note of the time needed for the color to recover once pressure is released. CRT reflects the time for blood refilling the peripheral vascular bed; studies have shown that CRT is related to perfusion. Normal CRT is usually less than 2 s. Abnormal prolonged value of more

than 4.5 s has the clinical implications, suggesting that insufficient peripheral tissue perfusion. There are studies suggesting that mottling score and CRT are well correlated with abdominal organ perfusion [11].

In addition, *the forearm-to-fingertip skin-temperature gradient* (Tskin-diff) can also be used to assess peripheral perfusion [11].

Although clinical parameters such as decreased urine output, skin hypoperfusion prolonged CRT, and fluctuating mental status changes are instructive in indicating hypoperfusion [4], improvement in these signs cannot reflect the improvement in tissue perfusion promptly and accurately. In particular, it is less time-sensitive than hemodynamic parameters and therefore cannot be used as a goal to direct the fluid resuscitation.

11.4 Pressure Parameters

Pressure parameters include artery pressure (AP), right atrium pressure (RAP), central venous pressure (CVP), pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PAWP), etc.

11.4.1 AP

AP is a parameter that often used by clinicians for fluid resuscitation because it is very easy to get. For critically ill patients, ambulatory blood pressure monitoring helps to keep abreast of changes of disease and direct the resuscitation. For those patients who require vasoactive drugs for septic shock, an initial mean arterial pressure (MAP) target of 65 mmHg or more is currently recommended to ensure perfusion of vital organs [12]. However, the baseline of AP is affected by many factors such as age, gender, mental status, underlying diseases, and posture. Therefore, when AP is the goal of resuscitation, individual differences should also be noticed in patients. For example, patients with underlying chronic hypertension may need higher blood pressure as the goal of resuscitation [13]. In addition, AP is also susceptible to measurement methods: The measured value of invasive AP and noninvasive AP will be somewhat different, and the distance of measured site to the heart also can influence the measurement results [14].

11.4.2 CVP and RAP

CVP is the pressure in the right atrium or in the vena cava near the right atrium. It is an important parameter that reflects blood volume and right ventricular function. CVP is usually measured by the central venous catheter placed in subclavian vein or internal jugular vein. The normal CVP is 4–12 cmH_2O; it reflects the changes of blood volume in the body, volume of venous return, right ventricular filling pressure, and right ventricular function. CVP reflects the right atrial pressure (RAP),

their values are usually similar, and thus CVP is usually used as a surrogate for RAP. CVP is affected by three factors—the right ventricle systolic performance, circulatory blood volume, and systemic vascular tone. CVP is a common hemodynamic parameter for it can be easily and continuously monitored. When the cardiac function is relatively normal, CVP is a good indicator to reflect the preload and cardiac function and determine the resistance to venous return. Maintaining a normal CVP level is necessary to ensure adequate venous return and cardiac output. The "2–5 cmH_2O" principle to judge volume load was used to direct the fluid therapy in clinical practices. (After the rapid fluid resuscitation, if the CVP elevates no more than 2 cmH_2O, it indicates that continuation of infusion is needed. If CVP elevates more than 5 cmH_2O, then infusion should be stopped. If CVP elevates between 2 and 5 cmH_2O, then it should be carefully assess whether the infusion should be continued or not.) Studies have shown that high CVP is a major factor related to the organ perfusion because organs blood flow is driven by the pressure difference between the arteries and veins. The effects of high CVP on the kidneys are particularly obvious. Increased venous pressure leads to increased pressure under the kidney capsule, which reduced renal blood flow and then decreased glomerular filtration rate. Therefore, excessively high CVP (>12 mmHg) reminds clinicians at this time to carefully assess whether fluid resuscitation should be performed in patients with septic shock or not.

Whether CVP is a good goal for fluid resuscitation has been controversial for long time: Many factors influence measured CVP, such as posture, catheters, intrathoracic pressure, intra-abdominal pressure, etc. In a systematic review of CVP, the area under the ROC curve for CVP to predict fluid responsiveness was only 0.5, suggesting that CVP cannot predict fluid responsiveness. Therefore, the guideline recommends that CVP cannot be used alone to direct fluid resuscitation, especially if its value is still relatively normal (8–12 mmHg) [12].

11.4.3 Pulmonary Artery Wedge Pressure (PAWP)

PAWP can be measured through the pulmonary artery catheter: After inflating the balloon at the tip of the pulmonary artery catheter, the distal balloon wedged into a small pulmonary arterial branch of the pulmonary artery, the normal value of the measured balloon pressure is 6–12 mmHg. When PAWP meet the measurement requirements, it has a correlation with left atrial pressure and left ventricular end-diastolic pressure. Meanwhile, the blood flow in arterial system is depended on left ventricular output, while left ventricular output is correlated with left ventricular preload. Therefore, PAWP can reflect the left ventricular preload. For example, increased PAWP is the earliest manifestation of the decline in ventricular function. Similar to the clinical implications of CVP, excessive PAWP (>12 mmHg) also reminds clinicians to be cautious in assessing the use of fluid resuscitation in patients with septic shock. PAWP is also affected by many factors, such as cardiac compliance, changes in intrathoracic pressure, and so on, so dyspnea or mechanical ventilation significantly affects PAWP.

In addition, pulmonary artery pressure (PAP) is also a common monitoring parameter of pulmonary artery catheter and is used to assess the afterload on the right ventricle and reflect pulmonary vascular resistance.

11.5 Volume Parameters

Volume parameters include the global end-diastolic volume index (GEDVI), intrathoracic blood volume index (ITBVI), right ventricular end-diastolic volume index (RVEDVI), left ventricular end-diastolic volume index (LVEDVI), etc. Compared with the pressure parameters, the volume parameters can more directly reflect the changes in the length of the cardiac muscle to reflect the preload. In recent years, with the development of clinical technology, preload/volume monitoring is more widely used. For example, GEDVI and ITBVI can be measured by placement of a pulmonary artery catheter or Pulse Indicator Continuous Cardiac Output (PiCCO) technique, both of which have been shown to better reflect preload than PAWP and CVP.

Pressure parameters and volume parameters are static parameters, but available evidences show that static parameters have a poor performance to predict the fluid responsiveness. This is because that the cardiac filling pressure is the pressure in the cavity, while the preload is determined by the transmural pressure, which is influenced by both the intracavity pressure and the extracavity pressure. Moreover, the preload parameter alone also cannot predict fluid responsiveness, because the patient's fluid responsiveness is determined by the patient's preload and cardiac contractility. The preload parameters can predict the fluid responsiveness only when the ventricular contractility is normal. Therefore, the above preload parameters cannot be used to predict fluid responsiveness. However, when the static preload parameters are at the upper or lower limit of its normal range, it still has some practical aspect for directing fluid resuscitation: If the parameter is at the lower limit of the normal range, it indicates that infusion may be safe, and if it is at the upper limit of normal range, the decision of infusion should be careful.

11.6 Blood Flow Parameters

Blood flow parameters include CO, SV, etc. To exclude the influence by the size of body, the above parameters were divided by the body surface area and converted into CI and SVI. Blood flow parameters are important for hemodynamics management and are instructive in directing fluid resuscitation and even administration of positive inotropic agents or vasoactive drugs. Currently, the above parameters are available in a variety of ways, including invasive or noninvasive monitoring, but the gold standard is still available through monitoring with either the pulmonary artery catheter or pulse-indicated continuous cardiac output (PICCO). Real-time monitoring of the above flow parameters can be used to direct the decision of continued fluid resuscitation in patients, the most commonly clinically application is the fluid

responsiveness test. The scientific concept behind fluid resuscitation is to improve cardiac output (CO) or stroke volume (SV), thus improve organ perfusion, and correct organ dysfunction. Fluid resuscitation increases cardiac output only when the following two conditions are met: (1) The increased amplitude of the mean capillary filling pressure (MCFP) is higher than the increased amplitude of CVP in rapid rehydration, thereby increasing the pressure gradient for venous return; (2) Ventricular function is in the ascending portion of the Frank-Starling curve. In these circumstances, the patient's response to fluid resuscitation is fluid responsiveness. Studies show that only about 50% of hemodynamically unstable patients have fluid responsiveness. Rapid fluid resuscitation should only be performed in the patient who has fluid responsiveness and may benefit from it. When fluid responsiveness disappears, patients should no longer undergo rapid fluid resuscitation.

11.6.1 Fluid Challenge

It is the most commonly clinical method to assess the fluid responsiveness. After a rapid infusion of 250–500 mL of fluid to patients within 15–30 min, a fluid responsiveness is indicated if the patient's CO or SV is increased by more than 10–15%, indicating that infusion may be continued. If not, infusion should be restricted. The gold standard to assess fluid responsiveness is the change in SV after the rehydration test. The risk of a fluid responsiveness test is that too much fluid may be infused into patients with no fluid responsiveness in the test.

11.6.2 Mini Fluid Challenge

It is a modified fluid responsiveness test. After a rapid infusion of 100 mL of colloids to patients in 1 min, the fluid responsiveness is indicated if the patient's SV is increased by more than 5% [15, 16]. Despite mini fluid challenge reduced infusion volume, similar risks of fluid responsiveness test cannot be avoided.

11.6.3 Passive Leg Raise Test (PLR)

The original head up semi-recumbent position was adjusted by changing the position of the bed, lowering patient's upper body to horizontal, and passively raising legs at 45° up. So systemic pressure is increased, thereby venous return is increased. Due to the gravity, PLR can induce additional 300–500 mL blood from lower extremities and abdominal cavity flow back to the heart. During this process, a real-time measurement of changes of cardiac output or stroke volume in a short period of time (<5 min, usually within 1–2 min) is conducted. PLR is positive if cardiac output or stroke volume increases more than 10–15% and indicates that these patients with septic shock can be considered with fluid responsiveness and can resume fluid resuscitation [17]. The advantage of PLR is that its effect of

increasing the blood volume is reversible without additional fluid infusion, thereby avoiding to infuse unnecessary fluid volumes to patients without fluid responsiveness. PLR is suitable for patients with spontaneous breathing and patients with arrhythmia or low tidal volume ventilation. The type of mechanical ventilation and the type of resuscitation fluid do not affect its diagnostic performance [18, 19]. However, the test is not suitable for patients with leg amputations, certain urological or gynecological procedures, and patients with traumatic brain injuries or intracranial hypertension [20].

Every time before infusion, its potential benefits and risks should be assessed. Fast infusion should only be performed when the patient has fluid responsiveness and may benefit from it. While it should not be performed when fluid responsiveness disappears.

11.7 Metabolic Parameters

Metabolic parameters include mixed venous oxygen saturation (SvO2), central venous oxygen saturation (ScvO2), lactate (LAC), and venous-to-arterial carbon dioxide difference (PvCO2-PaCO2, ΔPv-aCO2).

11.7.1 Mixed Venous Oxygen Saturation (SvO2)

SvO2 reflects the relationship between oxygen consumption and oxygen delivery in the body; it can be used to determine systemic tissue perfusion and the body's oxygen uptake and consumption. Its value is directly related to cardiac output, hemoglobin, arterial partial pressure of oxygen, and arterial oxygen saturation. Its value is inversely related to the body's metabolic rate. The normal SvO2 is 75%, SvO2 > 65% indicates proper oxygen reserve, SvO2 of 50–60% indicates limited oxygen reserve, and 35–50% indicates insufficient oxygen reserve. Central venous oxygen saturation (ScvO2) is usually used as a surrogate for SvO2 since SvO2 can only be measured by placing a pulmonary artery catheter. ScvO2 is usually not equal to SvO2, but its change level and direction are basically the same as those of SvO2. A decreased SvO2 indicates that oxygen consumption exceeds oxygen delivery and increased oxygen uptake. An increased SvO2, especially >80%, indicates increased oxygen delivery or decreased oxygen consumption or decreased tissue oxygen utilization. Thus, ScvO2 monitoring may help diagnose the etiology of shock and predict outcome trends in some patients, but a normal level of SvO2 as a goal of therapy does not improve morbidity or mortality in critically ill patients. SvO2 in patients with septic shocks is often greater than 70% due to depletion of oxygen uptake capacity and microcirculatory shunting in the peripheral tissues, and a very high SvO2 (>90%) is associated with poor prognosis. Severe septic shock complicated with cardiac dysfunction will induce low SvO2, suggesting that we need to improve tissue perfusion. Due to the limitations of ScvO2 and SvO2

mentioned above, when they are normal or higher than normal, they cannot reflect the patient's volume status; at this time other parameters need to be used to assess tissue perfusion, such as central venous-to-arterial carbon dioxide partial pressure difference, respiratory quotient, etc. [21].

11.7.2 Lactate (LAC) and Lactate Clearance

Normal blood lactate level is 1 mmol/L, and blood lactate >2 mmol/L mostly indicates the imbalance between supply and demand of oxygen and the increasing anaerobic metabolism. Mortality in patients with severe sepsis complicated with lactic acidosis is increased, whereas those who are not complicated with lactic acidosis have a better prognosis. Therefore, considering that elevated lactate is the result of tissue hypoxia and inadequate oxygen delivery, the restore of lactate to normal levels may be beneficial in patients with septic shock, so new guideline recommend lactate as an indicator for fluid resuscitation in patients with septic shock [22].

In the initial stage of shock, lactic acidosis is in accord with the systemic hemodynamics status, and the higher the elevated lactate, the worse hemodynamics is. Under such circumstances, decreasing lactate rapidly as a goal to direct fluid resuscitation of shock is effective. However, in late-stage shock, even if systemic hemodynamics is stable, microcirculatory dysfunction in tissues may still exist [23]. The lactate metabolic process becomes slow, which may affect the accuracy of lactate goal-directed fluid resuscitation.

Compared with lactate level, lactate clearance rate is considered more accurate by many researchers. After fluid resuscitation, a decrease in elevated lactate concentrations in patients with septic shock suggests a better prognosis, possibly reflecting the effectiveness of fluid resuscitation. However, the level of lactate depends on the production and metabolism, not just the clearance [24]. And the increase of lactate is affected by many factors, and clinicians need to be aware of other causes of lactic acidosis. For example, the increased blood lactate concentration following the administration of epinephrine in patients with septic shock, on the contrary, is associated with higher survival rates. This is because that adrenaline increases oxygen delivery, but at the same time, it increases glycolysis due to catecholamines, which results in an increase of blood lactate. In addition, impaired liver function will affect the level of lactate metabolism, leading to accumulation of lactic acid.

11.7.3 Venous-to-Arterial Carbon Dioxide Difference (PvCO2-PaCO2, ΔPv-aCO2)

Venous-to-arterial carbon dioxide difference is also a parameter of tissue perfusion. The increased ΔPv-aCO2 in patients with septic shock reflects the decreased blood flow rate and hypoperfusion, and the tissue-produced CO2 cannot be passed

back the pulmonary circulation in time. When ScvO2 is abnormal, increased ΔPv-aCO2 mainly reflects the reduction of cardiac output. When ScvO2 is higher than normal, increased ΔPv-aCO2 may reflect the hypoperfusion of microcirculation. In patients with septic shock, PvaCO2 >6 mmHg in patients with corrected normal ScvO2 indicates a poor prognosis. Changes in PvaCO2 within the first 6 h after ICU admission can provide more information. In patients with an initial PvaCO2 >6 mmHg, if the PvaCO2 return to normal after treatment, the mortality will be reduced.

11.7.4 Venous-to-Arterial Carbon Dioxide Difference/Arterial-to-Venous Oxygen Content Difference (ΔPv-aCO2/ΔCa-vO2)

Respiratory quotient is the ratio of CO2 produced to O2 consumed. According to the Fick equation, this ratio can be calculated as cardiac output × venoarterial difference in CO2 concentration/cardiac output × arteriovenous difference in oxygen concentration; it can be simplified as venoarterial difference in CO2 concentration/arteriovenous difference in O2 concentration. Because the blood CO2 concentration is proportional to the partial pressure of CO2, while the partial pressure of CO2 is easier to be measured, thus the equation is converted to venous-to-arterial carbon dioxide difference/arterial-to-venous oxygen content difference (ΔPv-aCO2/ΔCa-vO2). This ratio predicts the appearance of VO2/DO2 dependency, and the ratio >1.6 may also be associated with poor prognosis [25].

Therefore, both lactate level and central venous oxygen saturation have limitations in assessing whether oxygen delivery is sufficient, a strategy combining both of them may be of good direction. The mortality rates for the following four conditions are from low to high: normal lactate with decreased ScvO2, elevated lactate with decreased ScvO2 (early stage), elevated lactate with decreased ScvO2 (delayed), and elevated lactate with elevated ScvO2. Therefore, lactate clearance and central venous oxygen saturation should be combined with other tests such as echocardiography and ΔPv-aCO2 to better understand the cause, severity, and prognosis of shock.

11.8 Dynamic Parameters

Due to the limitations of traditional static pressure and volume parameters, the choice of dynamic parameters that can accurately assess the patient's cardiac function is particularly important in directing fluid resuscitation for patients with septic shock [26]. Dynamic hemodynamic monitoring is based on the principle of heart-lung interaction [27]. It takes the degree of the circulatory system influenced by the respiratory motion as a parameter to predict the response of the circulatory system to the fluid load and then to dynamically monitor the status of the circulatory volume. The dynamic parameters for determining fluid responsiveness include stroke volume variation (SVV), the pulse pressure variation (PPV), end-expiratory occlusion test (EEOt), superior/inferior vena cava collapsibility index (SVC-CI, IVC-CI), etc.

11.8.1 Stroke Volume Variation (SVV)

SVV refers to the ratio of difference between maximum SV and minimum SV to mean SV during three respiration cycles at least or within 30s; it is based on the changes in intrathoracic pressure which will lead to elevated SV during inspiratory and decreased SV during expiratory. In patients with hypovolemia, SVV induced by mechanical ventilation is more obvious. A SVV greater than 13% can indicate the fluid responsiveness in patients with septic shock. However, it should be noted that SVV has predictive value only in mechanically ventilated patients with adequate sedation, without spontaneous breathing, and absence of arrhythmia. Also too high or too low tidal volume in mechanical ventilation can affect its accuracy.

11.8.2 Pulse Pressure Variation (PPV)

Pulse pressure is the difference between the systolic and diastolic pressure in one heartbeat (pulse pressure = systolic pressure—diastolic pressure), and pulse pressure variation (PPV) is another semi-invasive monitoring method that has been shown to be able to predict the fluid responsiveness. PPV is measured by determining the variation of the maximum pulse pressure and minimum pulse pressure during at least three respiratory cycles or within 30 s. PPV > 13–15% suggests that patients with septic shock have fluid responsiveness. Like SVV, PPV has predictive value only in mechanically ventilated patients with adequate sedation, without spontaneous breathing, and absence of arrhythmia. Too high or too low tidal volume in mechanical ventilation can affect its accuracy.

11.8.3 End-Expiratory Occlusion Test (EEOt)

During mechanical ventilation, each inspiratory process increases intrathoracic pressure and reduces venous return. Therefore, press the expiratory hold button for 15 s in the expiratory phase to eliminate the effect of increased intrathoracic pressure on venous return during inspiration. Thus it increases preload and achieves similar effects as rehydration tests and can assess patient's fluid responsiveness by monitoring changes in cardiac output. In general, EEOt > 10–15% is considered that the patients have fluid responsiveness. EEOt is still valid for patients with arrhythmia, but only for mechanically ventilated patients with adequate sedation, without spontaneous breathing. And too high or too low tidal volume in mechanical ventilation also can affect its accuracy.

11.8.4 Superior/Inferior Vena Cava Collapsibility Index (SVC-CI, IVC-CI)

Superior/inferior vena cava collapsibility index (SVC-CI, IVC-CI) is the variation of superior and inferior vena cava (SVC and IVC) diameters measured by transesophageal echocardiogram (TEE) or transthoracic echocardiogram (TTE) during

the respiratory cycle. The variations of vena cava (SVC and IVC) diameters can reflect the response to fluid therapy and volume status of circulatory system. Because SVC-CI needs to be measured through TEE examination, its clinical application is relatively limited. While IVC-CI can be obtained through TTE, which is more easy to perform in clinical practices, so the IVC-CI is more widely used. IVC-CI > 18% in patients with mechanical ventilation is considered to be with fluid responsiveness. While for spontaneous breathing patients, IVC-CI > 50% is considered to be with fluid responsiveness. It should be noted that the sonography is limited by the operator's personal experience. And the patient's body condition and abdomen condition also affect the image quality. For patients with dyspnea, increased intra-abdominal pressure, or other conditions with significant intra-abdominal pressure changes, measurement of IVC-CI should be cautiously applied to assess the fluid responsiveness.

11.8.5 Tidal Volume Challenge

Patients with septic shock are often complicated with acute respiratory distress syndrome (ARDS), whereas the low tidal volume ventilation strategy results in not accurately predicting patient's fluid responsiveness by PPV and SVV. While the fluid responsiveness can be predicted by monitoring the changes of PPV, SVV through increasing the patient's tidal volume from 6 mL per kilogram of ideal body weight to 8 mL/kg of ideal body weight for 1 min. A >3.5% absolute increase of PPV, a greater than 48% relative increase of PPV, a greater than 2.5% absolute increase of SVV, or a greater than 43% relative increase of SVV indicates that a patient has fluid responsiveness. Tidal volume challenge avoids the limitation of predicting fluid responsiveness in patients with low tidal volume ventilation by PPV and SVV. However, spontaneous breathing and arrhythmia still affect the accuracy of tidal volume challenge [28].

11.9 Conclusion

In 2001, Rivers presented the early goal-directed therapy (EGDT) strategies that continuously monitor some physiological parameters such as central venous pressure (CVP), mean arterial pressure (MAP), and central venous oxygen saturation (ScvO2) to direct intravenous infusion for septic shock patients, thus optimize oxygen delivery to tissues, and improve the patients' outcome [29]. As a result, early goal-directed therapy for sepsis management (resuscitation bundle) is recommended by the Surviving Sepsis Campaign. Recently three trials (ProCESS, ARISE, and ProMISe) question the continued need for all of the elements of early goal-directed therapy or the need for protocolized care for patients with severe and septic shock [30–32]. Nevertheless, EGDT still provides us with a good strategy for fluid resuscitation of septic shock management: To find the right goal for septic shock to direct fluid resuscitation. However, it must be recognized that due to the

pathophysiological characteristics of septic shock, the goals of resuscitation at different stages of shock are different. Therefore, in the continuous process of the development of septic shock, we must fully understand the meaning and limitations of various parameters, so as to select the right goal to direct the fluid resuscitation therapy.

References

1. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, et al. Developing a new definition and assessing new clinical criteria for septic shock: for the third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315:775–87.
2. Chugh SN, Malhotra KC. Acute pericarditis in aluminium phosphide poisoning. J Assoc Physicians India. 1992;40:564.
3. Guerin L, Monnet X, Teboul JL. Monitoring volume and fluid responsiveness: from static to dynamic indicators. Best Pract Res Clin Anaesthesiol. 2013;27:177–85.
4. Postelnicu R, Evans L. Monitoring of the physical exam in sepsis. Curr Opin Crit Care. 2017;23:232–6.
5. Lima A, Bakker J. Clinical assessment of peripheral circulation. Curr Opin Crit Care. 2015;21:226–31.
6. Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, et al. Acute kidney injury in sepsis. Intensive Care Med. 2017;43:816–28.
7. Abahuje E, Munyaneza R, Riviello R, Ntirenganya F. Assessment of hemodynamic response to fluid resuscitation of patients with intra-abdominal sepsis in low- and middle-income countries. J Surg Res. 2017;218:162–6.
8. Egal M, Erler NS, de Geus HR, van Bommel J, Groeneveld AB. Targeting oliguria reversal in goal-directed hemodynamic management does not reduce renal dysfunction in perioperative and critically ill patients: a systematic review and meta-analysis. Anesth Analg. 2016;122:173–85.
9. Kramer GC, Luxon E, Wolf J, Burnett DR, Nanduri D, Friedman BC. Inaccuracy of urine output measurements due to urinary retention in catheterized patients in the burn ICU. J Burn Care Res. 2017;38:e409–409e417.
10. de Moura EB, Amorim FF, da Cruz Santana AN, Kanhouche G, de Souza Godoy LG, de Jesus Almeida L, et al. Skin mottling score as a predictor of 28-day mortality in patients with septic shock. Intensive Care Med. 2016;42:479–80.
11. Brunauer A, Kokofer A, Bataar O, Gradwohl-Matis I, Dankl D, Bakker J, et al. Changes in peripheral perfusion relate to visceral organ perfusion in early septic shock: a pilot study. J Crit Care. 2016;35:105–9.
12. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit Care Med. 2017;45:486–552.
13. Asfar P, Meziani F, Hamel JF, Grelon F, Megarbane B, Anguel N, et al. High versus low blood-pressure target in patients with septic shock. N Engl J Med. 2014;370:1583–93.
14. Chemla D, Antony I, Zamani K, Nitenberg A. Mean aortic pressure is the geometric mean of systolic and diastolic aortic pressure in resting humans. J Appl Physiol (1985). 2005;99:2278–84.
15. Goodwin K, Abrahamowicz M, Leonard G, Perron M, Richer L, Veillette S, et al. Dietary vitamin a and visceral adiposity: a modulating role of the retinol-binding protein 4 gene. J Nutrigenet Nutrigenomics. 2015;8:164–73.
16. Strucinska M, Rowicka G, Dylag H, Riahi A, Bzikowska A. Dietary intake of vitamin D in obese children aged 1-3 years. Rocz Panstw Zakl Hig. 2015;66:353–60.

17. Monnet X, Teboul JL. Passive leg raising: keep it easy. Intensive Care Med. 2010;36:1445; author reply 446
18. Cherpanath TG, Hirsch A, Geerts BF, Lagrand WK, Leeflang MM, Schultz MJ, et al. Predicting fluid responsiveness by passive leg raising: a systematic review and meta-analysis of 23 clinical trials. Crit Care Med. 2016;44:981–91.
19. Monnet X, Marik P, Teboul JL. Passive leg raising for predicting fluid responsiveness: a systematic review and meta-analysis. Intensive Care Med. 2016;42:1935–47.
20. Monnet X, Teboul JL. Passive leg raising: five rules, not a drop of fluid. Crit Care. 2015;19:18.
21. Monnet X, Julien F, Ait-Hamou N, Lequoy M, Gosset C, Jozwiak M, et al. Lactate and venoarterial carbon dioxide difference/arterial-venous oxygen difference ratio, but not central venous oxygen saturation, predict increase in oxygen consumption in fluid responders. Crit Care Med. 2013;41:1412–20.
22. Jansen TC, van Bommel J, Schoonderbeek FJ, Sleeswijk VSJ, van der Klooster JM, Lima AP, et al. Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. Am J Respir Crit Care Med. 2010;182:752–61.
23. Kiyatkin ME, Bakker J. Lactate and microcirculation as suitable targets for hemodynamic optimization in resuscitation of circulatory shock. Curr Opin Crit Care. 2017;23:348–54.
24. Vincent JL, Orbegozo CD, Acheampong A. Current haemodynamic management of septic shock. Presse Med. 2016;45:e99–99e103.
25. Perner A, Gordon AC, De Backer D, Dimopoulos G, Russell JA, Lipman J, et al. Sepsis: frontiers in diagnosis, resuscitation and antibiotic therapy. Intensive Care Med. 2016;42:1958–69.
26. Marik PE, Monnet X, Teboul JL. Hemodynamic parameters to guide fluid therapy. Ann Intensive Care. 2011;1:1.
27. Michard F, Teboul JL. Using heart-lung interactions to assess fluid responsiveness during mechanical ventilation. Crit Care. 2000;4:282–9.
28. Myatra SN, Prabu NR, Divatia JV, Monnet X, Kulkarni AP, Teboul JL. The changes in pulse pressure variation or stroke volume variation after a "tidal volume challenge" reliably predict fluid responsiveness during low tidal volume ventilation. Crit Care Med. 2017;45:415–21.
29. Almesri N, Das NS, Ali ME, Gumaa K, Giha HA. Independent associations of polymorphisms in vitamin D binding protein (GC) and vitamin D receptor (VDR) genes with obesity and plasma 25OHD3 levels demonstrate sex dimorphism. Appl Physiol Nutr Metab. 2016;41:345–53.
30. Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, et al. Goal-directed resuscitation for patients with early septic shock. N Engl J Med. 2014;371:1496–506.
31. Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, et al. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014;370:1683–93.
32. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, et al. Protocolised Management In Sepsis (ProMISe): a multicentre randomised controlled trial of the clinical effectiveness and cost-effectiveness of early, goal-directed, protocolised resuscitation for emerging septic shock. Health Technol Assess. 2015;19:i–xxv, 1–150

Secondary Infection in Sepsis: Clinical Significance, Immune Mechanism, and Therapy Strategies

12

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Abstract

Sepsis is a common and main cause of morbidity and mortality in intensive care units and emergency departments. Recent evidence illustrated that patients who are suffering from sepsis undergo a prolonged immunosuppressive phase. As a consequence, many septic patients are at risk for secondary infection which is considered to be the major reason for the high mortality of this disease nowadays. In this paper, we discuss the clinical significance of secondary infection and its potential immune mechanisms. In addition, the conventional measures and novel immunomodulatory strategies are also summarized.

Keywords

Sepsis · Secondary infection · Nosocomial infection · Immune Immunosuppression

Sepsis is an uncontrolled inflammatory response caused by infection. The estimated mortality of sepsis is 10–20%, which can reach up to 60% when shock is present. The large number of inflammatory mediators in sepsis contributes to shock and multiple organ dysfunction syndrome (MODS) in sepsis. Nevertheless, many recent studies have demonstrated that sepsis is associated with only a transient hyperinflammatory phase. Subsequently, patients enter a prolonged immunosuppressive phase. As a result, patients who survive the acute phase of sepsis are at high risk of secondary nosocomial infection. The purpose of this paper is to discuss the current understanding of the clinical significance, mechanism, and treatment of secondary infection in sepsis.

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12.1 Secondary Infection in Sepsis

During the past decades, the knowledge about the basic pathophysiologic processes of sepsis has been improved rapidly which contributes to the development of treatment strategies of sepsis. The first Surviving Sepsis Campaign (SSC) guideline for the management of severe sepsis and septic shock was published in 2004 and has been updated every 4 years. Due to these efforts, the outcomes of sepsis have been improved year by year. Recent evidence illustrated that the absolute mortality rate of patients with severe sepsis decreased from 35% in 2000 to 18.4% in 2012 [1]. A meta-analysis of 36 multicenter data also found that severe sepsis mortality decreased from 46.9% in 1991–1995 to 29% in 2006–2009 [2]. However, unlike other diseases, such as acute myocardial infarction and stroke, the mortality of sepsis still remains unacceptably high. More importantly, from 1997 to 2010, septic shock patients with hospital mortality decreased by only 9% [3]. Similar results were also reported by Goto et al. [4]. Until now, sepsis is still one of the main causes of death in clinical critically ill patients.

According to the recent evidence, about 70% of septic death occurred 3 days after admission. In a study of 476 septic patients, 62.7% of total deaths occurred in the late phase of the disease [5]. As the early-phase mortality fell significantly, the sustained high mortality from severe sepsis and septic shock may be due to increased mortality in the late phase of the disease [5]. It has been demonstrated that more than 80% of the deceased septic patients had signs of continuous infections and the rates of common opportunistic bacteria and fungi increased significantly in the late phase (>15 days) of severe sepsis and septic shock when compared with the early phase (<6 days) of the disease [6]. Additionally, 39% of septic shock patients who survived the early phase (<3 days) of the disease developed secondary nosocomial infection in the ICUs [5]. In addition to bacterial and fungi infection, a study observed that over 40% of septic patients had reactivation of latent herpes viruses [7]. Moreover, the risk of late death for septic shock patients with secondary infection was about 5.8 times higher than that for patients without [5]. Another retrospective study also found that septic shock patients who died more than 3 days after ICU admission were related to hospital-acquired complications, including secondary infections [8]. Recently, secondary infection has been considered as the major cause of death for patients with sepsis [9, 10].

12.2 Immune Dysfunction and Secondary Infection Post-sepsis

Our previous study found that age, the severity of the disease, invasive medical measures, and length of ICU hospital stay were associated with secondary infection in septic shock patients [5]. However, it is well known that pathogens cause disease, not only dependent on the pathogenicity of pathogens but also closely related to the immune function of the host. Evidence showed that patients admitted with severe sepsis were more susceptible to nosocomial infection than other ICU patients

without sepsis and sepsis is an independent risk factor for nosocomial infection in ICU patients [11]. It has been recognized that the unique immune status of sepsis patients may influence their susceptibility to secondary infection.

12.2.1 Innate Immune Defects

12.2.1.1 Neutrophils

The innate immune system, also known as the non-specific immune system, is the first line of host defense against pathogenic organisms. The innate immune system consists of physical epithelial barriers, phagocytic cells, and circulating plasma molecules. Neutrophils comprise the largest number of the main innate immune cells in the body, and the majority of them remain housed in the bone marrow or immune centers. Together with macrophages, neutrophils constitute the professional phagocytes, and they have a primary role in protecting the host from pathogen invasion. Neutrophils kill or remove pathogenic microorganisms through the oxygen-dependent and the oxygen-independent mechanisms. In the oxygen-dependent process, killing was mediated by oxygen free radicals and other reactive oxygen species (ROS) generated by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex in response to pathogens. It has been reported that there was a significant reduction in the respiratory burst of BAL neutrophils from septic animals challenged with *P. aeruginosa* when compared to sham-treated mice [12]. Interestingly, similar to septic mice, the production of ROS in neutrophils in NADPH-deficient mice was also decreased, and an increased susceptibility to *P. aeruginosa* infection was also observed [12]. So, neutrophil deficiency may contribute to the secondary infection post-sepsis.

12.2.1.2 Monocytes and Macrophages

It has been observed that, similar to the phenomenon of endotoxin tolerance, the cytokine production capacity of monocytes and macrophages from septic mice and humans in response to LPS was severely reduced [13]. This phenomenon is a crucial pathophysiological adaptation to regulate overexuberant inflammation. On the other hand, it also contributes to immunosuppression after sepsis. Previous evidence illustrated that the levels of human leukocyte antigen (HLA)-DR on macrophages from sepsis were significantly decreased and were associated with the outcomes as well as the incidence of secondary infection [14, 15]. Additionally, the macrophages from septic patients secrete high levels of IL-10 which plays an important role in inhibiting the activity of Th1 cells, macrophages, as well as NK cells. As a result, the susceptibility of host to secondary infection in sepsis was increased [14, 15]. In addition, the expression of IL-1 receptor-associated kinase (IRAK)-M in macrophages was upregulated in sepsis, and the expression of the Toll-like receptor (TLR)-4 was reduced, which contribute to the decreased expression of pro-inflammatory cytokine and phagocytic function of macrophages [16]. The restored pathogen clearance ability of macrophages and increased survival rate of septic animals were observed after inhibiting IRAK-M expression [16].

12.2.1.3 Dendritic Cells

Dendritic cells (DCs), originating from $CD34^+$ hematopoietic stem cells, are one of the most powerful types of antigen-presenting cells (APCs). Mature DCs express major histocompatibility complex (MHC) molecules, co-stimulatory molecules (CD40, CD80, CD83, and CD86), and adhesion molecules and migrate to lymphoid organs and induce specific immune response of T cells [17]. A large number of previous studies have confirmed that the number of DCs in sepsis was significantly decreased and the percentage of mature DCs also trended toward reduced levels [17]. In addition, the ability of DCs to secrete pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-12, and IL-1β is reduced in sepsis, whereas the production of anti-inflammatory cytokines including IL-10 and transforming growth factor (TGF)-β was significantly increased [17, 18]. IL-2 is a key molecule for T-cell proliferation and survival, and the ability of septic DC cells to induce proliferation and IL-2 production of T cells were reduced. Adoptive transfer of DCs from normal mice protected mice from secondary *P. aeruginosa* infection after sepsis, but the DCs from septic mice did not reduce the susceptibility of sepsis mice to secondary infection, suggesting that the decreased number and impaired function of DCs may be the crucial reasons of secondary infection in sepsis [19] (Fig. 12.1).

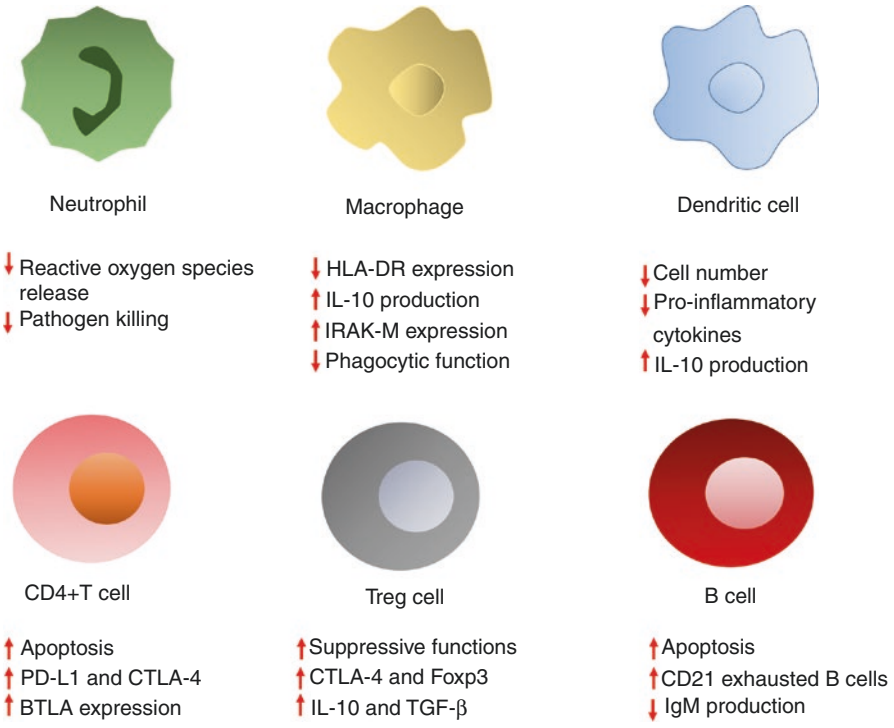

Fig. 12.1 Immune dysfunction associated with secondary infection in sepsis

12.2.2 Adaptive Immune Response Dysfunction

12.2.2.1 $CD4^+$T Lymphocytes

Cytokines and chemokines secreted by $CD4^+$T cells are crucial for the activation of macrophages and neutrophils. Additionally, the interactions between T cells and B cells are essential for the production of neutralizing antibodies to pathogens. Evidence have been illustrated that the proliferation of $CD4^+$T cells in response to polyclonal and antigen-specific stimulation was reduced [20]. A shift from Th1 to Th2 and increased apoptosis of T cells were also observed in sepsis [20]. Clinical studies illustrated that the number of circulating T cells was significantly decreased in septic patients and was positively associated with the illness severity and mortality [20]. In addition, surviving $CD4^+$T cells exhibited lower capability to secrete cytokines in response to LPS. Our previous studies found that mitofusin-2, a mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells, plays an important role in regulating $CD4^+$T cell immune function and apoptosis through $Ca2^+$-NFAT signaling pathway [21]. Recently, it has been found that increased levels of programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated antigen (CTLA)-4, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and B and T lymphocyte attenuator (BTLA) in $CD4^+$T cells might contribute to the defects of immune function of the cells in sepsis and the decrease in resistance to infectious pathogens in host survival [22, 23].

12.2.2.2 Regulatory T Cells

Regulatory T cells (Tregs), as one of the T-cell subsets, can be classified into natural Tregs (nTregs), adaptive/induced Tregs (iTregs), type 1 Tregs (Tr1), T helper 3 (Th3), and $CD8^+$Tregs. Forkhead/winged helix transcription factor p3 (Foxp3) is principally found within the $CD4^+CD25^+$Treg cell population and plays an important role in the development and functionality of these cells [24]. Tregs are crucial for modulating the immune responses in tumor immunity, transplantation tolerance, as well as infectious diseases. The suppressive function of Tregs is dependent on cell-cell contact mechanism and immunosuppressive cytokines [24]. Cytotoxic T lymphocyte antigen (CTLA)-4 and glucocorticoid-induced tumor necrosis factor receptor (GITR) mediate the function of cell contact-dependent suppression of Tregs [24]. CD25 on the surface of Tregs has high capacity to capture IL-2 which contributes to the suppressive function of the cells. Additionally, transforming growth factor (TGF)-β and interleukin (IL)-10 secreted by Tregs also play a role in immune depression [24].

Clinical studies found that the proportion of $CD4^+CD25^+$Treg in the peripheral blood of patients with sepsis is significantly increased [25]. Further studies illustrated that the absolute number of $CD4^+CD25^+$Treg cells did not increase significantly, but its immunosuppressive activity was enhanced [26, 27]. The expression of CTLA-4 and Foxp3 and the levels of IL-10 and TGF-β in Treg cells were significantly higher than those in the non-sepsis group [26, 27]. In addition, in patients with sepsis, the immunosuppressive function of Tregs is higher in the death group when compared with that in the survival group [26, 27]. Evidence illustrated DEREG mice depleted of $Foxp3^+$Treg cells before secondary infection with *P. aeruginosa* 7 days post-CLP had

no effects on the course of secondary infection as well as cytokine levels [28]. Nevertheless, our study found partial depletion of $CD25^{+}$Tregs by PC61 treatment 3 days post-CLP also enhanced host immune responses against secondary acute *P. aeruginosa* and improved the outcomes of the disease (unpublished data). The difference in the results might be explained on the basis of experimental techniques including septic models and the depletion strategies of Tregs. The exact role of Treg in secondary infection post-sepsis remains to be investigated.

12.2.2.3 B Lymphocytes

B lymphocytes arise from hemopoietic stem cells in the bone marrow which are characterized by their ability to differentiate into immunoglobulin secreting plasma cells. In addition to their role in humoral immunity, B cells have an important role in regulating immune homeostasis by their antigen-presenting and cytokine-producing capabilities [29]. Although there are some controversies, it is widely accepted that B cells act as effective antigen-presenting cells which are required for the initiation of T-cell immune responses during infection [29, 30]. Additionally, the bacterial products, such as LPS and CpG DNA, can cause the activation and cytokine production of B cells through Toll-like receptors (TLR) both in vivo and in vitro [31]. Recently, regulatory B cells, a phenotypically distinct subset of B cells, have been recognized as a crucial regulator of T-cell-mediated inflammatory responses through the production of IL-10 [32].

Previously study found that septic mice with adaptive immune system defects, including B cell apoptosis, display diminished survival. Nevertheless, the role of B cells in sepsis was not well examined. Rauch et al. [33] found that mice lacking B-cell-derived GM-CSF are unable to clear bacteria and succumb to infection. Kelly-Scumpia et al. [34] found that B-cell-deficient, but not α/β T-cell-deficient, mice display decreased inflammatory cytokine and chemokine production and increased mortality after sepsis. Clinical study has demonstrated that patients with septic shock suffer from a severe retraction of circulating B lymphocytes [35]. An exhausted B-cell phenotype has been associated with a $CD19^{+}CD5^{+}CD27^{-}CD21^{-/low}$ "tissue-like memory" B-cell subset in human immunodeficiency virus (HIV)-infected patients. Recently, it was reported that the fraction of CD21-exhausted B cells in sepsis patients was higher than that in healthy subjects [36]. Moreover, the IgM production was impaired in elderly septic patients, and this phenomenon was associated with increased susceptibility to secondary infection post-sepsis, as elderly septic patients showed a higher rate of *Candida albicans* and higher sputum colony counts than adult septic patients [36]. So, the defects of B lymphocyte function may be associated with immunosuppression and secondary infection in patients with sepsis.

12.3 Therapeutic Strategies for Secondary Infection

12.3.1 Conventional Strategies

According to the criteria of the Centers for Disease Control (CDC), there are four main types of nosocomial infections including central line-associated bloodstream

infections (CLABSI), surgical site infection (SSI), catheter-associated urinary tract infection (CAUTI), and ventilator-associated pneumonia (VAP). Prevention and control guidelines have been developed to reduce the incidence of these infections. The measures recommended by these guidelines, including the standard and transmission-based precautions as well as strategies focused on specific nosocomial infections, also can be applied to prevent and control secondary infection after sepsis [37]. It should be noted that secondary infection after sepsis has its own characteristics. Evidence illustrated that pneumonia was the most frequent secondary infection in septic shock patients and the most frequently isolated microorganism was *Acinetobacter baumannii* [5]. Additionally, as mentioned above, age, the SOFA score, length of stay in the ICU, and endotracheal intubation were observed to be associated with secondary infection post-sepsis [5]. So, improving the care of these patients may be useful for reducing the incidence of secondary infections after sepsis.

12.3.2 Immunomodulatory Therapy

12.3.2.1 Experimental Studies

Cytokines and Cytokine-Targeted Therapies

Cytokines are crucial regulators of the immune response to infections, and imbalance in inflammatory network has been found to be associated with the death of septic patients. Previous studies suggest that pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α, contributed to tissue damage and organ failure of sepsis [38]. Nevertheless, the failure of several anti-inflammatory clinical trials in sepsis has led researchers to re-recognize the function of pro-inflammatory cytokines. In fact, increased mortality was observed in TNF–/–LT–/– knockout mice with infection [39]. So, pro-inflammatory cytokines may be also important for protecting host from infections. In contrast to pro-inflammatory cytokines, anti-inflammatory cytokines, such as IL-4 and IL-10, play a crucial role in inhibiting inflammation and contribute to immunosuppression in sepsis. Song et al. [40] found that IL-4-deficient mice that underwent CLP were resistant to secondary pulmonary *P. aeruginosa* infection which is characterized by better bacterial clearance and improved survival. Neutralization of TNF-α could reverse the enhanced protection against secondary infection in septic IL-4 KO mice, indicating the crucial role of TNF-α in this process [40]. Additionally, IL-10 and IL-27 were also observed to be associated with immunosuppression in sepsis, and neutralization of IL-10 or IL-27 could reverse sepsis-induced dysfunction of macrophages and improve both survival and clearance of bacteria from the lungs of septic mice infected with *P. aeruginosa* [41, 42].

IL-7, a 25-kDa glycoprotein produced by the bone marrow and thymic stromal cells, plays an important role in regulating T- and B-cell development and function [43–45]. Through its binding to a receptor (IL-7R), IL-7 can increase the levels of B-cell lymphoma 2 (BCL2) and the numbers of circulating $CD4^+$ and $CD8^+$T cells [46]. IL-7 also improves the ability of T cells to move to sites of infection by increasing the cell adhesion molecule expression. IL-7 treatment restores immunity and

improves survival in a viral model of lymphocytic choriomeningitis [47]. Previous studies have illustrated that rhIL-7 treatment inhibited the apoptosis of T lymphocyte by upregulating Bcl-2 expression and decreased the mortality of septic animals [48]. Additionally, in septic mice followed by *P. aeruginosa* and fungal infection, treatment with rhIL-7 could improve survival effectively [49]. More importantly, IL-7 treatment does not induce a hyper-inflammatory response. IL-7 is currently undergoing numerous clinical trials, including in patients with septic shock (ClinicalTrials.gov identification # NCT02640807, # NCT02797431).

In addition to IL-7, IL-15 is also under consideration for treating sepsis. Studies have shown that IL-15 could inhibit the apoptosis of NK cells, DC cells, and $CD8^+$T cell in sepsis [50]. In mice with peritonitis or *P. aeruginosa* pneumonia, IL-15 improved T lymphocyte survival, enhanced the capacity of cell to secrete cytokines, and ultimately decreased the mortality of the animals [50]. However, IL-15 did not improve the capacity of mice to clear bacteria [50]. Additionally, it should be noted that IL-15 superagonist has potential hepatotoxicity and its clinical safety remains scarce [51–53].

Immune Checkpoint Therapies

Immune checkpoints are molecules in the immune system that are crucial for maintaining self-tolerance. The CTLA-4 and PD-1 immune checkpoints are negative regulators of the immune function of T cells [54–56]. PD-1, a newly discovered co-stimulatory receptor, belongs to the CD28 superfamily of receptors [54–56]. PD-L1 and PD-L2 are the main ligands of PD-1. PD-L1 is widely expressed on dendritic cells, macrophages, and activated T lymphocytes and B lymphocytes [54–56]. Increased levels of PD-L1 on T cells and monocytes were observed in septic patients [57–59]. Studies also illustrated that PD-L1 can be used as a predictive and prognostic marker for sepsis. Blocking PD-L1 can reduce TNF-α and anti-CD3/anti-CD28 antibody-induced $CD4^+$T and $CD8^+$T cell apoptosis and can promote the expression of IL-6, TNF-α, and IFN-γ in monocytes and lymphocytes [58–60]. CTLA-4, as an analogue of CD28, has a higher binding activity to B7.1 and B7.2 [54]. Nevertheless, binding of CTLA-4 to B7 does not produce a simulator signal. So, it has been proposed that CTLA-4 dampens the activation of T cells by outcompeting CD28 in binding B7. In addition, evidence also illustrated that CTLA-4 binding to B7 on T cells also actively delivers inhibitory signals to the T cells, blocks TCR activating, and then inhibits T-cell proliferation [54]. Animal studies found that blocking PD1/PD-L1 and CTLA-4 improved the outcomes of sepsis or sepsis with secondary fungal infection [23]. Recently, nivolumab and pembrolizumab, both antibodies against PD-1, received FDA approval for the treatment of patients with unresectable or metastatic melanoma as well as metastatic squamous and nonsquamous non-small cell lung cancer (NSCLC) [61]. However, the clinical evidence of immune checkpoint therapies in sepsis are still lacking and need further investigation.

B and T lymphocyte attenuator (BTLA) is a co-inhibitory receptor which is expressed on B and T lymphocytes, macrophages, dendritic cells, as well as NK cells [62]. The ligand of BTLA is herpes virus entry mediators (HVEM) that belong

to tumor necrosis factor receptor (TNFR) family [62]. BTLA has potential role in inhibiting $CD4^+$T cell and B-cell function and diminishing pro-survival signaling in $CD4^+$T cells [62]. Clinical studies have shown that the levels of BTLA on peripheral blood $CD4^+$T cells in patients with sepsis are significantly higher than that in patients without sepsis [63]. Additionally, in patients admitted with SIRS, BTLA could serve as a biomarker of hospital infection [63]. However, another clinical study found that the number of $BTLA^+CD4^+$T cells in patients with sepsis was significantly lower than that of healthy volunteers at 24 hours after hospitalization and it was lower in dead patients when compared with survivors [64]. The different results may be due to the different stages of the disease. In addition to BTLA, V-domain immunoglobulin suppressor of T-cell activation (VISTA), T-cell immunoglobulin- and mucin-domain-containing molecules (Tim), and lymphocyte activation gene-3 (LAG-3) also have potential role in modulating host immune function and are expected to be new immune therapy targets of sepsis [65–67].

Other Strategies

Due to the advances in the understanding of immune pathophysiology of sepsis, many immunomodulatory drugs or agents have been discovered and used in preclinical studies. Caspase inhibitors and TAT-conjugated anti-apoptotic Bcl-2-like peptides have been proved to inhibit immune cell apoptosis in septic animals [68]. The immunomodulatory effects of Xuebijing and *Astragalus* polysaccharide, as traditional Chinese medicines, have been illustrated by some studies [69, 70]. Ethyl pyruvate (EP) is a simple ramification derived from the pyruvic acid and has been shown to be an experimental therapeutic on immune dysfunction. Our recent study have found that EP treatment protected septic mice from secondary *P. aeruginosa* pneumonia and the protective effects of EP may via decreasing lung IL-10 and plasma HMGB1 expression, inhibiting the function of Tregs and relieving the apoptosis of splenic immune cells [71] (Table 12.1).

12.3.2.2 Clinical Trials

IFN-γ

IFN-γ is a member of the type II IFN family. Although mice deficient of IFN-γ and its receptor (IFN-γR) are resistant to LPS-induced toxicity, previous studies showed that IFN-γ is also crucial for an effective host response to variety of pathogens including virus and bacteria [72, 73]. Additionally, evidence also illustrated that INF-γ receptor mutations are associated with the increased host susceptibility to infections [72, 74]. Moreover, an important study found that, in septic patients with low monocytic HLA-DR expression, IFN-γ treatment restored the deficient HLA-DR expression and in vitro LPS-induced TNF secretion [75]. Recovery of monocyte function resulted in clearance of sepsis in eight of nine patients [75]. In addition, in severe trauma patients with less than 30% HLA-DR expression on alveolar macrophages, about 50% of patients had elevated levels of HLA-DR in macrophages after INF-γ inhalation, and the incidence of hospital-acquired pneumonia in these patients was significantly reduced [76]. Although this study included

Table 12.1 Immunomodulatory therapeutic strategies for secondary infection in sepsis

	Evidence	References
Cytokines		
IL-4	IL-4-deficient mice that underwent CLP were resistant to secondary pulmonary *P. aeruginosa* infection	[40]
IL-10	Neutralization of IL-10 improves both survival and clearance of bacteria from the lungs of septic mice infected with *P. aeruginosa*	[41]
IL-27	Neutralization of IL-27 improves both survival and clearance of bacteria from the lungs of septic mice infected with *P. aeruginosa*	[42]
IL-7	rhIL-7 improves survival in septic mice followed by *P. aeruginosa* or fungal infection	[49]
IFN-y	INF-y inhalation decreases the incidence of hospital-acquired pneumonia in severe trauma patients with immune dysfunction	[76]
GM-CSF	GM-CSF treatment reduces the incidence of nosocomial infection in children with MODS	[80]
Immune checkpoints	Blockade of PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis	[23]
Others		
Ethyl pyruvate	The susceptibility of septic mice to secondary *P. aeruginosa* pneumonia is downregulated by ethyl pyruvate treatment	[71]

only 21 patients, the results suggested the potential value of INF-γ in preventing secondary infection trauma and sepsis [76]. Recently, a phase III clinical trial investigating the role of IFN-γ in sepsis-induced immune suppression and secondary infection is ongoing (ClinicalTrials.gov identification # NCT01649921).

G-CSF and GM-CSF

The colony-stimulating factors (CSFs) comprise a group of cytokines including granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF). As other CSFs, G-CSF and GM-CSF are crucial for the hematopoiesis of blood cells, the maintenance of homeostasis, and immune competence [77]. In the past few decades, there are many trials investigating the effect of G-CSF and GM-CSF as potential adjunctive immunomodulatory agents in patients with sepsis. It has found that G-CSF and GM-CSF treatment reduced the duration of mechanical ventilation and hospital stay in severe sepsis and septic shock patients with low HLA-DR levels [78]. In non-traumatic abdominal infection patients, GM-CSF could reduce the complications of infection and the length of hospital stay [79]. In addition, GM-CSF treatment has been shown to reduce the incidence of nosocomial infection in children with MODS [80]. However, recent meta-analysis found that G-CSF and GM-CSF failed to reduce the overall mortality of sepsis patients [81]. Because most trails included in the meta-analysis did not stratify study patients according to their immunological state and the effect of G-CSF and GM-CSF on the function of immune system was not reported, the exact effect of G-CSF or GM-CSF therapy in sepsis requires further evaluation.

Thymosin Alpha1

Thymosin alpha1 (Tα1) is a thymus-derived immunomodulatory peptide which acts as an endogenous regulator of immune systems [82]. It has reported that Tα1 played a unique role in maintaining the balance of pro- and anti-inflammatory cytokine production [82, 83]. Tα1 has been widely used in clinical trials for the treatment of disease associated with immune dysfunction [84, 85]. The efficiency of Tα1 in treating chronic B and C hepatitis as well as some types of cancers has been proved [84, 86]. Previous animal study found that Tα1 could improve the survival of septic mice [87]. Clinical trial also found that Tα1 treatment increased the mHLA-DR levels and decreased the mortality in septic patients [88]. As Tα1 has shown immune-enhanced effects in both animal and clinical studies, the effect of it on secondary infection due to immunosuppression in sepsis deserves further attention.

12.4 Conclusions

Due to the advance in early goal-directed therapy and new antibiotic and adjunct strategies, more and more septic patients survive the phase of acute circulation failure and organ dysfunction and enter a prolonged immunosuppressive state which is characterized by the defects of both innate and adaptive immune responses. Secondary infection is a clinical manifestation of immune dysfunction in sepsis and contributes to poor outcomes of the patients. Recently, in addition to conventional strategies, the effect of immunomodulatory therapy in preventing secondary infection after sepsis has been proved by both preclinical studies and few clinical trials. These efforts may help to reduce the incidence of secondary infection in sepsis and further reduce the mortality of the disease.

References

1. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012. JAMA. 2014;311(13):1308–16.
2. Stevenson EK, Rubenstein AR, Radin GT, et al. Two decades of mortality trends among patients with severe sepsis: a comparative meta-analysis. Crit Care Med. 2014;42(3):625–31.
3. Walkey AJ, Wiener RS, Lindenauer PK. Utilization patterns and outcomes associated with central venous catheter in septic shock: a population-based study. Crit Care Med. 2013;41(6):1450–7.
4. Goto T, Yoshida K, Tsugawa Y, Filbin MR, Camargo CA Jr, Hasegawa K. Mortality trends in U.S. adults with septic shock, 2005–2011: a serial cross-sectional analysis of nationally-representative data. BMC Infect Dis. 2016;16:294.
5. Zhao GJ, Li D, Zhao Q, Song JX, et al. Incidence, risk factors and impact on outcomes of secondary infection in patients with septic shock: an 8-year retrospective study. Sci Rep. 2016;6:38361.
6. Otto GP, Sossdorf M, Claus RA, Rödel J, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. Crit Care. 2011;15(4):R183.

7. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. PLoS One. 2014;9(2):e98819.
8. Daviaud F, Grimaldi D, Dechartres A, et al. Timing and causes of death in septic shock. Ann Intensive Care. 2015;5(1):16.
9. Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? J Clin Invest. 2016;126(1):23–31.
10. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013;13(3):260–8.
11. León C, Ruiz-Santana S, Saavedra P, et al. A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. Crit Care Med. 2006;34(3):730–7.
12. Delano MJ, Thayer T, Gabrilovich S, et al. Sepsis induces early alterations in innate immunity that impact mortality to secondary infection. J Immunol. 2011;186(1):195–202.
13. López-Collazo E, del Fresno C. Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences. Crit Care. 2013;17(6):242.
14. Lekkou A, Karakantza M, Mouzaki A, et al. Cytokine production and monocyte HLA-DR expression as predictors of outcome for patients with community-acquired severe infections. Clin Diagn Lab Immunol. 2004;11(1):161–7.
15. Lukaszewicz AC, Grienay M, Resche-Rigon M, et al. Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. Crit Care Med. 2009;37(10):2746–52.
16. Deng JC, Cheng G, Newstead MW, et al. Sepsis-induced suppression of lung innate immunity is mediated by IRAK-M. J Clin Invest. 2006;116(9):2532–42.
17. Fan X, Liu Z, Jin H, Yan J, Liang HP. Alterations of dendritic cells in sepsis: featured role in immunoparalysis. Biomed Res Int. 2015;2015:903720.
18. Luan YY, Dong N, Xie M, et al. The significance and regulatory mechanisms of innate immune cells in the development of sepsis. J Interf Cytokine Res. 2014;34(1):2–15.
19. Pène F, Zuber B, Courtine E, Rousseau C, et al. Dendritic cells modulate lung response to *Pseudomonas aeruginosa* in a murine model of sepsis-induced immune dysfunction. J Immunol. 2008;181(12):8513–20.
20. Cabrera-Perez J, Condotta SA, Badovinac VP, et al. Impact of sepsis on CD4 T cell immunity. J Leukoc Biol. 2014;96(5):767–77.
21. Zhao GJ, Yao YM, Lu ZQ, et al. Up-regulation of mitofusin-2 protects CD4+ T cells from HMGB1-mediated immune dysfunction partly through Ca(2+)-NFAT signaling pathway. Cytokine. 2012;59(1):79–85.
22. Arens C, Bajwa SA, Koch C, et al. Sepsis-induced long-term immune paralysis—results of a descriptive, explorative study. Crit Care. 2016;20:93.
23. Chang KC, Burnham CA, Compton SM, et al. Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. Crit Care. 2013;17(3):R85.
24. Corthay A. How do regulatory T cells work? Scand J Immunol. 2009;70(4):326–36.
25. Monneret G, Debard AL, Venet F, et al. Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. Crit Care Med. 2003;31(7):2068–71.
26. Cavassani KA, Carson WF 4th, Moreira AP, Wen H, Schaller MA, Ishii M, Lindell DM, Dou Y, Lukacs NW, Keshamouni VG, Hogaboam CM, Kunkel SL. The post sepsis-induced expansion and enhanced function of regulatory T cells create an environment to potentiate tumor growth. Blood. 2010;115(22):4403–11.
27. Huang LF, Yao YM, Dong N, Yu Y, He LX, Sheng ZY. Association between regulatory T cell activity and sepsis and outcome of severely burned patients: a prospective, observational study. Crit Care. 2010;14(1):R3.
28. Tatura R, Zeschnigk M, Hansen W, et al. Relevance of $Foxp3^+$ regulatory T cells for early and late phases of murine sepsis. Immunology. 2015;146(1):144–56.
29. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008;112(5):1570–80.

30. Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature. 1985;314:537–9.
31. Browne EP. Regulation of B-cell responses by toll-like receptors. Immunology. 2012;136(4):370–9.
32. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity. 2015;42(4):607–12.
33. Rauch PJ, Chudnovskiy A, Robbins CS, et al. Innate response activator B cells protect against microbial sepsis. Science. 2012;335(6068):597–601.
34. Kelly-Scumpia KM, Scumpia PO, Weinstein JS, et al. B cells enhance early innate immune responses during bacterial sepsis. J Exp Med. 2011;208(8):1673–82.
35. Monserrat J, de Pablo R, Diaz-Martín D, et al. Early alterations of B cells in patients with septic shock. Crit Care. 2013;17(3):R105.
36. Suzuki K, Inoue S, Kametani Y, et al. Reduced Immunocompetent B cells and increased secondary infection in elderly patients with severe Sepsis. Shock. 2016;46(3):270–8.
37. Mehta Y, Gupta A, Todi S, et al. Guidelines for prevention of hospital acquired infections. Indian J Crit Care Med. 2014;18(3):149–63.
38. Chaudhry H, Zhou J, Zhong Y, et al. Role of cytokines as a double-edged sword in sepsis. In Vivo. 2013;27(6):669–84.
39. Netea MG, van Tits LJ, Curfs JH, et al. Increased susceptibility of TNF-alpha lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of *Candida albicans*. J Immunol. 1999;163(3):1498–505.
40. Song Z, Zhang J, Zhang X, et al. Interleukin 4 deficiency reverses development of secondary *Pseudomonas aeruginosa* pneumonia during sepsis-associated immunosuppression. J Infect Dis. 2015;211(10):1616–27.
41. Steinhauser ML, Hogaboam CM, Kunkel SL, et al. IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense. J Immunol. 1999;162(1):392–9.
42. Cao J, Xu F, Lin S, et al. IL-27 controls sepsis-induced impairment of lung antibacterial host defence. Thorax. 2014;69(10):926–37.
43. Namen AE, Lupton S, Hjerrild K, et al. Stimulation of B-cell progenitors by cloned murine interleukin-7. Nature. 1988;333(6173):571–3.
44. Hand TW, Morre M, Kaech SM. Expression of IL-7 receptor alpha is necessary but not sufficient for the formation of memory CD8 T cells during viral infection. Proc Natl Acad Sci U S A. 2007;104(28):11730–5.
45. Corfe SA, Paige CJ. The many roles of IL-7 in B cell development; mediator of survival, proliferation and differentiation. Semin Immunol. 2012;24(3):198–208.
46. Sheikh V, Porter BO, DerSimonian R, Kovacs SB, et al. Administration of interleukin-7 increases CD4 T cells in idiopathic CD4 lymphocytopenia. Blood. 2016;127(8):977–88.
47. Audigé A, Hofer U, Dittmer U, et al. Evaluation of the immunomodulatory and antiviral effects of the cytokine combination IFN-α and IL-7 in the lymphocytic choriomeningitis virus and friend retrovirus mouse infection models. Viral Immunol. 2011;24(5):375–85.
48. Unsinger J, McGlynn M, Kasten KR, et al. IL-7 promotes T cell viability, trafficking, and functionality and improves survival in sepsis. J Immunol. 2010;184(7):3768–79.
49. Shindo Y, Fuchs AG, Davis CG, et al. Interleukin 7 immunotherapy improves host immunity and survival in a two-hit model of *Pseudomonas aeruginosa* pneumonia. J Leukoc Biol. 2017;101(2):543–54.
50. Inoue S, Unsinger J, Davis CG, et al. IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis. J Immunol. 2010;184(3):1401–9.
51. Waldmann TA, Lugli E, Roederer M, et al. Safety (toxicity), pharmacokinetics, immunogenicity, and impact on elements of the normal immune system of recombinant human IL-15 in rhesus macaques. Blood. 2011;117(18):4787–95.
52. Wege AK, Weber F, Kroemer A, et al. IL-15 enhances the anti-tumor activity of trastuzumab against breast cancer cells but causes fatal side effects in humanized tumor mice (HTM). Oncotarget. 2017;8(2):2731–44.
53. Guo Y, Luan L, Rabacal W, et al. IL-15 superagonist-mediated immunotoxicity: role of NK cells and IFN-γ. J Immunol. 2015;195(5):2353–64.

54. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. Am J Clin Oncol. 2016;39(1):98–106.
55. Callahan MK, Postow MA, Wolchok JD. CTLA-4 and PD-1 pathway blockade: combinations in the clinic. Front Oncol. 2015;4:385.
56. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25(21):9543–53.
57. Shao R, Fang Y, Yu H, et al. Monocyte programmed death ligand-1 expression after 3-4 days of sepsis is associated with risk stratification and mortality in septic patients: a prospective cohort study. Crit Care. 2016;20(1):124.
58. Zhang Y, Li J, Lou J, et al. Upregulation of programmed death-1 on T cells and programmed death ligand-1 on monocytes in septic shock patients. Crit Care. 2011;15(1):R70.
59. Chang K, Svabek C, Vazquez-Guillamet C, et al. Targeting the programmed cell death 1: programmed cell death ligand 1 pathway reverses T cell exhaustion in patients with sepsis. Crit Care. 2014;18(1):R3.
60. Zhang Y, Zhou Y, Lou J, et al. PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. Crit Care. 2010;14(6):R220.
61. Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? J Immunother Cancer. 2016;4:48.
62. Murphy KM, Nelson CA, Sedý JR. Balancing co-stimulation and inhibition with BTLA and HVEM. Nat Rev Immunol. 2006;6(9):671–81.
63. Shubin NJ, Monaghan SF, Heffernan DS, et al. B and T lymphocyte attenuator expression on CD4+ T-cells associates with sepsis and subsequent infections in ICU patients. Crit Care. 2013;17(6):R276.
64. Shao R, Li CS, Fang Y, et al. Low B and T lymphocyte attenuator expression on CD4+ T cells in the early stage of sepsis is associated with the severity and mortality of septic patients: a prospective cohort study. Crit Care. 2015;19:308.
65. Ren F, Li J, Jiang X, et al. Plasma soluble Tim-3 emerges as an inhibitor in sepsis: sepsis contrary to membrane Tim-3 on monocytes. Tissue Antigens. 2015;86(5):325–32.
66. Nowak EC, Lines JL, Varn FS, et al. Immunoregulatory functions of VISTA. Immunol Rev. 2017;276(1):66–79.
67. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity. 2016;44(5):989–1004.
68. Hotchkiss RS, McConnell KW, Bullok K, et al. TAT-BH4 and TAT-Bcl-xL peptides protect against sepsis-induced lymphocyte apoptosis in vivo. J Immunol. 2006;176(9):5471–7.
69. Liu YC, Yao FH, Chai YF, et al. Xuebijing injection promotes M2 polarization of macrophages and improves survival rate in septic mice. Evid Based Complement Alternat Med. 2015;2015:352642.
70. Liu QY, Yao YM, Yu Y, et al. Astragalus polysaccharides attenuate postburn sepsis via inhibiting negative immunoregulation of CD4+CD25(high) T cells. PLoS One. 2011;6(6):e19811.
71. Chen W, Lian J, Ye JJ, et al. Ethyl pyruvate reverses development of Pseudomonas aeruginosa pneumonia during sepsis-induced immunosuppression. Int Immunopharmacol. 2017;52:61–9.
72. Car BD, Eng VM, Schnyder B, et al. Interferon gamma receptor deficient mice are resistant to endotoxic shock. J Exp Med. 1994;179(5):1437–44.
73. Romero CR, Herzig DS, Etogo A, et al. The role of interferon-γ in the pathogenesis of acute intra-abdominal sepsis. J Leukoc Biol. 2010;88(4):725–35.
74. Jouanguy E, Altare F, Lamhamedi S, et al. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. N Engl J Med. 1996;335(26):1956–61.
75. Döcke WD, Randow F, Syrbe U, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med. 1997;3(6):678–81.
76. Nakos G, Malamou-Mitsi VD, Lachana A, et al. Immunoparalysis in patients with severe trauma and the effect of inhaled interferon-gamma. Crit Care Med. 2002;30(7):1488–94.
77. Barreda DR, Hanington PC, Belosevic M. Regulation of myeloid development and function by colony stimulating factors. Dev Comp Immunol. 2004;28(5):509–54.

78. Meisel C, Schefold JC, Pschowski R, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med. 2009;180(7):640–8.
79. Orozco H, Arch J, Medina-Franco H, et al. Molgramostim (GM-CSF) associated with antibiotic treatment in nontraumatic abdominal sepsis: a randomized, double-blind, placebo-controlled clinical trial. Arch Surg. 2006;141(2):150–3.
80. Hall MW, Knatz NL, Vetterly C, et al. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. Intensive Care Med. 2011;37(3):525–32.
81. Bo L, Wang F, Zhu J, et al. Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: a meta-analysis. Crit Care. 2011;15(1):R58.
82. Romani L, Bistoni F, Montagnoli C, et al. Thymosin alpha1: an endogenous regulator of inflammation, immunity, and tolerance. Ann N Y Acad Sci. 2007;1112:326–38.
83. Romani L, Bistoni F, Perruccio K, et al. Thymosin alpha1 activates dendritic cell tryptophan catabolism and establishes a regulatory environment for balance of inflammation and tolerance. Blood. 2006;108(7):2265–74.
84. You J, Zhuang L, Cheng HY, et al. Efficacy of thymosin alpha-1 and interferon alpha in treatment of chronic viral hepatitis B: a randomized controlled study. World J Gastroenterol. 2006;12(41):6715–21.
85. Wang X, Li W, Niu C, et al. Thymosin alpha 1 is associated with improved cellular immunity and reduced infection rate in severe acute pancreatitis patients in a double-blind randomized control study. Inflammation. 2011;34(3):198–202.
86. Garaci E, Pica F, Rasi G, et al. Thymosin alpha 1 in the treatment of cancer: from basic research to clinical application. Int J Immunopharmacol. 2000;22(12):1067–76.
87. Wan J, Shan Y, Shan H, et al. Thymosin-alpha1 promotes the apoptosis of regulatory T cells and survival rate in septic mice. Front Biosci (Landmark Ed). 2011;16:3004–13.
88. Wu J, Zhou L, Liu J, et al. The efficacy of thymosin alpha 1 for severe sepsis (ETASS): a multicenter, single-blind, randomized and controlled trial. Crit Care. 2013;17(1):R8.

Research Progress of Burn Infection

13

Zichen Yang and Yizhi Peng

Abstract

Infection is one of the leading causes of death in severely burned patients. Because of the destruction of the physiological defense barrier in the body surface and the decline of systemic immune function in severe burn patients, the presence of a wide range of necrotic tissues and the invasion of the external and internal bacterial flora have increased the susceptibility to infection. So it can be said that the threat of local or systemic infection after burns starts from the burn injuries and ends till the wound heals.

13.1 Route of Burn Infection

13.1.1 Wound Surface

Wound surface is the main route of burn infection. Burn wound surface is not infected with many germs, due to high temperature injury [1]. However, bacteria quickly emerge and increase from the environmental contaminants, bacteria in remnant hair follicles and sweat glands, soon reproduce later in the wound surface rich of necrotic tissues, and invade through the damaged skin defense barrier. Thus, burn wound surface infection takes a high incidence rate and acts as a leading source of burn systemic infection. This burn complication usually takes place in burn wounds, especially deep burn wounds [2]. Burn wound infection is generally divided into noninvasive surface infection and invasive infection.

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Noninvasive Infection Along with liquefaction of necrotic tissue and formation of purulent secretions, wound surface bacteria load can be high. But once granulation tissue healthily forms at wound bottom, the necrotic tissue and purulent secretions can be drained and eliminated in time. The relatively high load of surface bacteria does not invade adjacent living tissue. And tissue culture of bacteria is often limited below "critical bacteria load" (1×10^5/g tissue) [3]. This type of burn surface infection belongs to noninvasive infection. Noninvasive infection lacks obvious systemic symptoms.

Invasive Infection Symptoms of invasive infection include wound surface getting wet, pus accumulated or foul smell, bleeding under wound surface or wound eschar, inflammatory infiltration around wound margin, and cellulitis. It even occurs with necrosis, wound surface growth arrest, and wound deepening when wound surface is severely infected. Second-degree burns can develop rapidly to third-degree burns. Granulation tissue can be reformed again to necrotic scab. Invasive infection is often accompanied by rapid deterioration of the whole-body situation. Once the invasive infection manifestation is clinically suspected, bacterial quantification of wound tissue should be applied to determine the situation. If wound surface bacteria count is $>1 \times 10^5$/g tissue, burn wound sepsis can be diagnosed [4].

13.1.2 Respiratory Tract

After severe burns, the respiratory tract can become an important way of systemic infection, especially in patients with inhalation injury or tracheotomy, to which enough attention should be paid. Inhalation injury causes airway mucosal edema, inflammatory exudation, and tissue necrosis and abscission, which often increases risks of obstructive atelectasis or pneumonia in these patients [5]. Gram-negative pathogen, which is prominent in burn infections, such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia*, *Klebsiella pneumonia*, *Escherichia coli* (*E. coli*), *Acinetobacter* species (spp.), etc., exists and breeds especially easy in such wet environment without special nutrition. In recent years, Gram-negative pathogen can often be clinically detected in the wide use of all kinds of inhalation devices, such as gas humidifier, atomizer, and humidification bottle in the oxygen supply device, produced by which small particles can reach the lower respiratory tract. If enough attentions are not paid to this, the respiratory tract can become an important route for the invasion of pathogens [6].

13.1.3 Intestinal Tract Infection

Notably, the intestinal tract is the largest "bacteria reservoir" and "endotoxin store" in the body, which makes it a potential infection route [7]. Damaged intestinal mucosal barrier, impaired immune functions and imbalanced gut microbiota in severe burns, allows intestinal bacteria and endotoxin to disseminate to the whole body through blood vessels and lymphatic vessels, to develop infection. This pathological process is called enterogenous infection.

13.1.4 Catheter-Related Infection

Catheter-related infection is an important route of iatrogenic infection. Venous catheters do not only cause phlebitis but also become an important source of systemic infection. Therefore, vein incision should be avoided if venipuncture is applicable; shallow veins should be used more while deep veins less; local disinfection and care should be paid attention to during intravenous infusion; and time of an indwelling catheter should be especially limited. Time of catheterization in wound surface should be less than 3–5 days and that in normal skin surface less than 7 days in burn patients. Once a nonfluency of infusion or fever of unknown origin (FUO) is found, extubation should be exerted resolutely, along with catheter tip microorganisms' culture [8, 9].

13.2 Enterogenous Infection of Burns

13.2.1 Proposition and Proof of the Problem

It is conventionally believed that pathogens of burn infection always arise from the wound to develop a so-called exogenous infection. In the year of 1962, we found positive blood culture emerged long before an identical wound culture in the 312 analyzed cases of burn patients with septicemia, among which culture results showed that bacteria were often intestinal residents. This finding indicated that enterogenous infection might be a long-undiscovered important issue in severe burn injury. At that time, aseptic and isolation technology have been developing for decades. However, it is still unacceptable and difficult to reduce surgical infection rate to a lower level. In addition, systemic infection often occurred with unknown primary infection source in clinical work. Thus, the research on burn infection redirected from the "exogenous" to "endogenous" route focusing on intestinal tract.

Many reasons contribute to this changing focus. Firstly, the gut is the largest reservoir of bacteria in the human body. The bacteria in the intestinal tract account for 78.7% of the total microbial population of a person [10]. In addition, the common bacteria in critically burned patients with secondary infection include not only *E. coli* but also *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus proteus*, *Acinetobacter* spp., and *Enterobacter cloacae*, all of which are intestinal residents. Since 1980, we have been conducting experimental studies on this problem, with more than 2000 experimental animals, including sterile and specific pathogen-free (SPF) rats. In the beginning, stress injury of the gastrointestinal tract after severe burn injury did not only occur in the stomach and duodenum (so-called curling ulcer) but also in the ileal mucosa along with obvious damage. Intestinal bacteria labeled (directly or indirectly) with fluorescein isothiocyanate (FITC), including aerobic bacteria (represented by *P. aeruginosa*), anaerobic bacteria (represented by *Bacteroides fragilis*), and fungi (represented by *Candida albicans*), were proved to invade through the intestinal tract and spread to the mesenteric lymph node, liver, spleen, lung, kidney, and blood, as early as 3 h after severe burn. The specific ways through which microbial penetrated intestinal mucosa were also

captured. In addition, enterogenous infection includes enterogenous endotoxin invasion. We have observed abnormal opening of enterogenous epithelial intercellular tight junction by freeze-etching technique and penetration of the abnormal open channel into the submucosal stroma by peroxidase staining. And detection proved that endotoxin level in portal vein blood indeed increased after burn injury. Furthermore, we studied the pathogenesis of enterogenous infection, aiming a series of secondary issues after severe burns by making models, respectively, and have got some acknowledgments.

(1) Hypotension (4 kPa) 30–60 min: enterogenous bacteria can invade the mesenteric lymph nodes; 190 min, enterogenous bacteria can widely invade the liver and spleen and blood; low blood volume time with enterogenous infection was positively correlated.
(2) Bacterial endotoxin, in return, can increase the permeability of the enterogenous mucosa.
(3) Protein malnutrition can lead to progressive enterogenous mucosal atrophy.
(4) Under the condition of worse enterogenous intestinal motility and enterogenous clogging, enterogenous microbiota changes with obvious proliferation of Gram-negative pathogen.

Above assaults continuously occurred after severe burn injury and mutually promote the occurrence and development of enterogenous infection. Moreover, Deitch mentioned that intestinal tract may be a potential way that enterogenous bacteria invade into the blood [11]; Marshall declared that enterogenous infection may be the trigger of multiple organ failure, reports on which suddenly increased in the 1990s [12]. Alexander referred that "the shift of enterogenous bacteria and endotoxin may be an important factor in immune suppression" [13]. Goodwins mentioned that intestinal tract might be the source of high metabolism after burn injury [14]. Meakins even mentioned that "intestinal tract is just like an abscess cavity without drainage under pathological state" [15]. So, the gut's potential route of infection after infection is worth noting.

13.2.2 Pathogenesis of Enterogenous Infection

The pathogenesis of enterogenous infection can be roughly divided into three main causes: (1) the microecological imbalance of enterogenous microbiota; (2) the breakdown of mucosal barrier in the intestinal tract; and (3) the inhibition of immune function [7]. To be specific:

Microecological Imbalance of Enterogenous Microbiota A normal flora is a micro ecosystem that is constituted by hosts and its microorganisms, including bacteria, fungi, protozoa, and other microorganisms, during historical coevolution. The normal flora is widely distributed in the body, among which the enterogenous microbiota has the greatest impact on the host. The intestine of a newborn person or animal is sterile; until 2–4 h later, the bacteria enter, settle, and multiply in it, which

is known as colonization. In the beginning, aerobic bacterium such as *E. coli* and *Enterococcus* colonized and then came the anaerobic bacteria. This process was completed within 1–2 weeks. The enterogenous microbiota (species and number of bacteria) is relatively stable after colonization unless there are external influencing factors. The enterogenous microbiota consists of at least 400–500 kinds of bacteria. There are 10^{11}–10^{12} bacteria in every gram of dry stool; among them, 90–99% are anaerobic bacteria. The enterogenous microbiota in the enterogenous cavity forms a multilevel biological layer: the deep layer consists mainly of parasitic anaerobic *Bifidobacteria* and *Lactobacillus*; the middle layer consists of *Bacteroides*, *Peptostreptococcus*, *Micrococcus*, and *Bacillus* superior; the surface layer consists of *Escherichia coli* and *Enterococcus*. Under an electron microscope, the deep layer's flora, which sticks to the surface of the enterogenous mucosa, is called membrane flora, coated by polysaccharide and relatively stable; the surface flora, which swims mainly in the enterogenous cavity, is called lumen flora. They form a complex ecological balance. There are important biological implications in host-enterogenous microbiota and inter-microbiota interactions. In the process of host growth and development, many physiological and immune functions perfect with the assistance of enterogenous microbiota. And the enterogenous microbiota is built on the mutual antagonism and coordination. The anaerobic membrane flora in the gut is a very important defense barrier of the host, named colonization resistance, which prevents the potentially pathogenic aerobic bacteria or invasive bacteria from colonizing on the mucous membrane. Once anaerobic bacteria reduced with colonization resistance impaired, aerobic bacteria can colonize or invade. The enterogenous microbiota is very sensitive and easily altered by the organic, functional, or mental changes in its hosts or the physical, chemical, or biological changes in the external environment. And the changes will cause a series of feedbacks to the host physiologically or pathologically including dysbacteriosis-induced light abdominal pain, diarrhea, or bacteria and endotoxin-induced severe enterogenous infection. Since no bacteria exist in germ-free animal, the relationship between opportunistic bacteria and enterogenous infection can be observed in the absence of colonization resistance in the gut. After the normal microbiota of SPF animal cecum was inoculated into germ-free animals' intestine, bacteria like *E. coli*, *Enterococcus*, *Klebsiella pneumoniae*, and *Mimea polymorpha* were isolated from mesenteric lymph nodes after a week from the germ-free animals by culture. After a single strain of *E. coli* was inoculated into the enterogenous tract of another group of germ-free animals, the same strain passage was isolated in 96% of the animals' mesenteric lymph nodes by culture after 2 weeks [16]. For normal animals, enterogenous inoculation of the abovementioned bacteria did not reproduce the same phenomenon. Some experimental studies have shown that among normal enterogenous anaerobic bacteria, *Clostridium* (fusiform group) play the key role in colonization resistance, which will release its metabolite volatile short-chain fatty acids to control the colonization of bacteria in the enterogenous cavity [17]. The mouse cecum is an important habitat site for anaerobic bacteria. After cecectomy, enterogenous *Clostridium* and volatile short-chain fatty acids were decreased, while bacteria amount of Gram-negative pathogen was increased by 1000 times. And the decline of enterogenous bacterial

colonization resistance allows intestinal pathogen not only to invade the mesenteric lymph nodes but also spread to the organs like the liver and spleen, etc. Long-term and large doses of broad-spectrum antibiotics are the most common causes of microbial disturbances in enterogenous microbiota, which have also been demonstrated in experimental studies. For instance, antibiotics like oral penicillin, clindamycin, and metronidazole can not only inhibit anaerobic bacteria but also elevate the number of bacteria of enterogenous Gram-negative pathogen 1000 times higher. After withdrawing of these drugs, the amount of Gram-negative pathogen decreased, and the process of pathogen invading to the body gradually stopped.

Damage of Enterogenous Mucosal Barrier Under physiological conditions, the amount of bacteria and endotoxin inside the intestinal lumen far exceeds the amount that is fatal to the host cells, which demands the intestinal mucosa to be an effective defensive barrier. When the intestinal mucosa is damaged for any cause, the bacteria and endotoxin may invade. In the case of severe burns, the early enterogenous infection is mainly caused by stress, which damages the intestinal mucosa. The damaged lesions are found not only in the upper digestive tract but also in the lower digestive tract where lies the largest bacteria reservoir inside the human body. In experimental studies, when 25% second-degree burn is imposed on the burn-sensitive Balb/c mice, the epithelium membrane of the intestinal mucosa is found falling and the mesenchyme exposed after 24 h. The bacteria's incidence of early invasion can reach as high as 74%. When the xanthine oxidase inhibitor allopurinol is applied to reduce the damages to the intestinal mucosa by curbing oxygen-free radicals, the incidence rate of pathogen invading can be lowered to 30%. Morehonse [18] reported that 4 h after the animals were fed with ricinoleic acid to cause damages to the intestinal mucosa, the bacteria in the intestinal canal were found not only in mesenteric lymph nodes but also in multiple organs like the liver and spleen. After the intestinal mucosa heals naturally, the bacteria's translocation was found stopped. Therefore, it can be concluded that the enterogenous infection occurs and develops in a fluctuated manner depending on the severity of damages to the intestinal mucosa barrier.

Inhibited Immune Functions After severe burns, the immunity functions including the cellular immunity and humoral immunity will be damaged. It has already been widely accepted that the damaged immunity is linked to the infection and its development. The enterogenous infection presents the following features: the disease-causing microorganisms are usually resident bacteria; when infection rises, the bacteria's toxicity almost remains the same level, while the host becomes more vulnerable. Among the several factors that lead to restricted immunity, the restriction of cell immunity has been well explained. The mice with congenital absence of the thymus and mice with deliberate removal of the thymus share the same enterogenous infection incidence rate of 50% respectively, which is a compelling evidence for enterogenous infection under conditions where only T cell's immunity weakens. When the mice are transplanted with thymus to regain T cell immunity, the incidence rate of enterogenous infection was lowered to 8% [13].

When the athymic mice are inflicted with 30% area superficial burns, the enterogenous bacteria are found to invade the liver, spleen, abdominal cavity, and blood system, which may develop fatal intestinal infection. Immunosuppression is reported to be closely associated with nutrition levels, especially the protein malnutrition. The protein malnutrition can lead to thymus contraction, which morphologically includes decreased lymphocyte, disappeared medulla differentiating areas, and Hassall body swelling. The T cell-dependent area, the peripheral lymphatic tissues and peripheral areas of artery in spleen tissues, is also found contracted. Besides, the weakened delayed-type hypersensitivity of the skin is also a manifestation of damaged cell immunity caused by malnutrition, which is attributed to changed number and function of T cell subset that can obstruct the antigen recognition and antigen-removing process.

Additionally, the phagocytes, neutrophils, and granulocytes are proved to be less effective in intracellular sterilization and removing Gram-negative pathogens. The adjusting capacity of serum antibody is weakened; the level of serum complement G3 is lower than the normal level; the respond ability to PHA of lymphocytes is weakened. The body's nonspecific defense function is also related to enterogenous infection. The main humoral immune components on internal and external surfaces are SIgAs that are produced 30–100 mg/kg per day by the human body. The mucous membrane covering the internal and external surface needs protection from SIgAs. The function of SIgA on intestinal mucosal surface is to prevent the intestinal flora from colonizing on the intestinal mucosa and to neutralize the toxins and enzymes in the intestinal cavities. Under physiological conditions, the caliciform cell of intestinal mucosa can produce mucus to form a viscoelastic gel layer on mucosal surfaces in which mucous glycoprotein is a key functional component; it can help the SIgA create an anti-infectious antibody-mucus barrier on mucosal surfaces to remove the bacteria and endotoxin in the mucous layer through normal intestinal peristalsis. Once the amount of mucus and mucoprotein decreases, the colonization resistance of intestinal mucosa is weakened, which increases the incidence rate of enterogenous infection.

To conclude, pathogenesis of enterogenous infection is a complex process, impeded by multiple factors. The newly proposed potential route of infection needs further exploration. By far, it has been recognized that the bacteria and endotoxin translocate themselves to intestinal lymphatic vessels, portal vein, and abdominal cavity after going through the intestinal mucosa barrier and further react with macrophage to produce large amount of cytokine (such as interleukin-1, interleukin-6, tumor necrosis factors) and arachidonic acid produces (such as thromboxane and prostaglandin). It can help produce neurohumor and stimulate the endocranial organs to produce a lot of cortisol, protocatechuic acid amine, and glucagon, which leads to clinical acute fever, impaired immunity, continuous ultrahigh metabolism, suppression of normal protein production and elevation of abnormal proteins, and extensive damages to viscera. Therefore, some researchers proposed that the intestinal tract may serve as the igniting organ for multiple organ failures.

13.2.3 Prevention and Treatment of Enterogenous Burn Infection

Study is few on prevention and treatment for enterogenous infection of burns. However, basic researches have proved that further knowledge about the disease will certainly generate new measures which may be helpful in handling the major challenges regarding the burn disease course.

Relation Between Burn Shock and Infection According to the shared experience among Chinese experts on burn injuries, patients with more severe shock period are vulnerable to septicemia. The internal relationship between shock and infection lacks explanations. Currently, it is widely accepted that the longer hypovolemic shock period results in more obvious translocation of enterogenous bacteria (including endotoxin) and higher detection rate of bacteria in blood and viscera. Due to multiple pathogenic factors (as mentioned above) after burn injury, the enterogenous infections during shock period are more probable, especially for patients suffering from large-area severe burns. Routine blood culture during burn shock period is seldom applied. In the early 1960s, the several positive blood cultures were found during burn shock in large-area burn patients during occasional blood culture. Recently, a study in Jinan Central Hospital of 11 large-area burn patients (average burn area is 81.5%, and third-degree burn area is 56.1%) has reported septicemia symptoms during burn shock period. The blood culture results in 72 h turned out to be uniformly positive, with the earliest positive result appearing in the first 18 h. Among the 13 strains of bacteria identified, the most dominant is the resident enterobacteria, while only 3 burn wounds identified the same strain. In the blood culture for the 14 burn patients in Xinxiang Second People's Hospital, the earliest positive result was identified 4 h after the burn. The most dominant bacteria are also the resident enterobacteria according to their analysis. Both the experimental research and clinical analysis indicate that the infections and shock are closely related in severe burn patients. Given the potential infectious factors that make the shock period more unstable, the sudden death caused by septicemia during shock period may largely be attributed to the larvaceous infection with a rather great severity. In traditional clinical practices, the burn course is usually divided to shock period and infection period [19]. However, for severely burned patients, shock and infection periods may overlay where lies a reciprocal causation relationship.

Clinical Manifestations of Early Enterogenous Infection It is a progressive process that the bacteria translocate to the mesenteric lymph node and viscera through the intestinal tract, to develop positive blood culture, which can be divided into three stages:

Subclinical infection: The bacteria's translocation is restricted to the mesenteric lymph node, which may be explained by the possible stalemate between the bacteria and the defensive function. During this process, the clinical manifestation is insignificant, including some fever, etc.

Toxemia: As the bacteria break the local defense barrier and invade the organs within the reticuloendothelial system like liver and spleen, the body will respond drastically

against the antigenic stimulus (caused by endotoxin or bacteria), including some cardiac and vascular system reactions. However, the shock period covers the reactions as aggravated shocks which defies improvement through volume supplementation. Though there are some signs of systematic infections including conscious disturbance and respiratory distress, no positive blood culture can be identified, while some tracer bacteria can be found in the viscus tissue culture in experimental animals.

Severe infection: As the defensive function of the intestinal mucosa and the reticuloendothelial system further deteriorates, the enterogenous bacteria and endotoxin will invade the blood circulation system to grow and reproduce. When the blood culture result turns positive, it can be viewed as severe infections. It occurs almost at the same stage when early fulminant sepsis appears.

Tips on Prevention and Treatment During Early Onset of Enterogenous Infection *Earliest anti-shock treatment*: As mentioned above, the hypovolemic shock is closely related to the incidence of enterogenous infection. Therefore, when dealing with severe burns, the priority should be volume supplementation against the shock, which is also significant for anti-infection treatment.

Systemic antibodies appliance: According to the results of animal experiments, the urgency (bacteria usually invade in 3 h, intestinal endotoxin even earlier) and the extensiveness (liver, spleen, lung, kidney, etc.) of enterogenous infection after severe burns indicate that anti-infection measures should be added along with the anti-shock treatment for large-area burn patients, especially those with delayed fluid infusion. Considering the high incidence rate and death rate of systemic infection among large-area burn patients, the short-term therapy for broad-spectrum treatment targeting the resident bacteria to which the intestinal tract is vulnerable, such as *E. coli*, *Serratia*, *P. aeruginosa*, *Proteus* spp., and *S. aureus*, should be rational.

According to studies of 312 burn septicemia patients, most septicemias (about 46.4%) are found in the first 3–7 days after burn. And 54 (74%) out of the 73 death cases occur during the first 3–7 days. In addition, Greenhalghet et al. reported several death cases result from septicemia [20]. Enterogenous infection is partially responsible for early-burn septicemia. The antibiotics present their best performance at the beginning of the bacteria's spreading, by maintaining a certain concentration of antibacterial drugs in tissues and blood. During the critical period of systemic infection, a short-term and systemic use of antibiotics has both prevention and treatment significance.

Early enteral feeding: In preliminary stages of severe burns, the intestinal peristalsis is often found to slow down. According to studies of animal models with intestinal obstruction, the Gram-negative pathogen can increase by 1000 times within 24 h. To prevent the intestinal tract from becoming a "physiological dead space," enteral feeding should be carried out at the earliest time, where even tiny amount of feeding can help improve the physiological function of the intestinal canal [21]. Besides, the feed ingredients may include some necessary amino acid for intestinal mucosal repair and some other ingredients, such as glutamine, that are difficult to be combined in nutritional formula.

Reduce stress injuries after severe burns.

13.3 Common Pathogens of Burn Infection

13.3.1 Gram-Negative Bacilli (G^- Bacilli)

Invasive burn wound infections are mostly caused by G^- bacilli. The microbes identified from the blood, tubes, and sub-scar tissues of the burn patients consist of 58.9% of G^-bacilli, 31% of G^+ cocci, and 10.1% of fungi. The cases of infection caused by G^- bacilli are two times as much as infections resulting from G^+ cocci. According to studies by burn injury institute of the Third Military Medical University, 3644 strains of G^- bacilli were identified in the 23,902 samples of burn patients collected from the year of 2006 to 2016 [2], as shown in Table 13.1.

As normal bacteria floras in human's intestinal tracts, the abovementioned strains identified from excrement examination make no clinical significance in normal situation. Therefore, they have long been considered as "nonpathogenic bacteria" or "opportunistic pathogen." Due to the strains' preferences for patients with immunodeficiency, clinical infections become increasingly dominant, especially among burn patients whose necrotic tissues offer breeding ground for saprophytic bacteria. Besides, the bacteria's natural resistance resulting from the antibiotics' filtering effects is also a contributing factor for the infections [22]. Despite their low toxicity, the bacteria prevail for their amounts through diffuse spread after going down through the eschars.

In terms of extensive severe burns, domestic treatments mainly include proper exposure and topical antibiotics (such as SD-Ag, etc.). However, topical antibiotics can only suppress the proliferation to buy time for escharectomy and skin grafting, with sub-scar bacteria amounts still increasing as disease courses prolong. Since the success of escharectomy and skin grafting is related to bacterial amounts in the sub-scar tissues which are relevant to disease courses, reliance on topical drugs should be rational. Therefore, early escharectomy and careful skin grafting are the fundamental approaches for the prevention and treatment of intrusive infections. While topical drugs merely serve as supportive measures.

Table 13.1 Common G^- bacilli in burn infection

Species	Strain	Detection rate (%)	Species	Strain	Detection rate (%)
Pseudomonas aeruginosa	1913	25.1	*Enterobacter cloacae*	283	7.8
Acinetobacter baumannii	730	20.0	*Proteus mirabilis*	1519	4.4
Escherichia coli	334	19.2	*Aeromonas hydrophila*	74	2.0
Klebsiella pneumoniae	323	8.7	*Serratia marcescens*	64	1.8

13.3.2 Gram-Positive Cocci (G^+ Cocci)

Currently, the G^+ cocci infections among burn patients are mostly caused by *Staphylococcus* and *Enterococcus*. Among the *Staphylococcus*, the *Staphylococcus aureus* is the most dominant. In the 23,902 samples of burn patients by the Third Military Medical University from 2006 to 2010, 1918 strains of Gram-positive cocci were detected, as shown in Table 13.2.

The *Staphylococcus aureus* is a common pathogen in burn infections. It cannot be thoroughly removed from the wound bed before the burn wound heals. In comparison with the G^- bacillus, the *Staphylococcus aureus* less cause burn wound sepsis yet can easily invade the blood system. For non-severe burn patients with small or medium area, the *Staphylococcus aureus* is the main pathogen. The sepsis caused by the *Staphylococcus aureus* usually presents the following symptoms: fever, overexcited mental disorder, enteroparalysis, and increased white cell counts.

Although the new semisynthetic penicillin was once considered as an effective controller for *Staphylococcus aureus*, the bacteria soon develop resistance against it. Actually, the methicillin-resistant *Staphylococcus aureus* (MRSA) is actually a multiple-resistant strain; it can also tolerate β-lactams and aminoglycosides. It is difficult to control the *Staphylococcus aureus* detected on durable burn wounds. The *Staphylococcus aureus* is an L-shaped coccus which is a member of abnormal bacteria. It survives after its cell wall structures are partially broken by the antibodies and continue to grow under proper osmotic pressure in the purulent exudates. It retains toxicity, and cannot grow and be detected on conventional medium. Only on hypertonic medium can they be identified.

The *Staphylococcus epidermidis* is a type of coagulase-negative *Staphylococcus*. In conventional bacterial examination, the reaction of plasma-clotting enzyme is an indicator to determine whether the *Staphylococcus* is pathogenic or not. Since the *Staphylococcus epidermidis* reacts with negative results and is a kind of resident bacteria of the human body, it has long been considered as a nonpathogenic *Staphylococcus*. However, in recent years, the infections resulting from the *Staphylococcus* are rising. The *Staphylococcus epidermidis* can produce a lot of

Table 13.2 Common G^+ cocci in burn infection

Species	Strain	Detection rate (%)	Species	Strain	Detection rate (%)
Staphylococcus spp.			*Enterococcus* spp.		
Staphylococcus aureus	1187	61.19	*Enterococcus faecium*	171	8.19
Staphylococcus haemolyticus	1190	10	*Enterococcus faecalis*	130	6.8
Staphylococcus epidermidis	87	4.5			
Staphylococcus hominis	64	3.3			

mucinous substances where the bacteria can stick and live to avoid the body's defensive reactions. Besides, according to our drug-sensitivity tests, the *Staphylococcus epidermidis* have higher drug resistance than the *Staphylococcus aureus*, which should be paid serious attention.

13.3.3 Fungi

Candida Candida infections are mostly found after the occurrence of other bacterial infections. It can be largely categorized as superinfection resulting from microbial ecological imbalance. It usually coexists with the original bacteria, which makes it more difficult to be differentiated. Additionally, as suitable breeding grounds for general bacteria, the conventional culture medium doesn't always fit for all fungi [23].

Clinical manifestations:

- Protracted development, less sharp than bacteria-caused infections; poor response to general antibiotics.
- Constant shifts between consciousness and unconsciousness in the patients.
- Frequent oral ulcer.
- Patients are easily chocked when feeding or have dysphagia.
- Mild fever with accelerated breath and heart rate.
- Patients with candida gastrointestinal ulcers can produce mucus-like melena.
- Tongue is less red than that of patients with bacteria-caused infections.
- When the infection invades the respiratory tract, the patients can produce gelatinous tissue blocks where candida can be identified through special culture.
- Sudden spasmodic respiration, urgent incision for air tube*s* is needed to ease the dyspnea.

Rather than relying on routine bacteria culture, special culture medium (such as Sabouraud medium) should be prepared for laboratory diagnosis. The infection can be attributed to microbial imbalance. Despite venous catheterization caused by candida, for most patients, candida growth can also be found at resident flora sites like the mouth, pharynx, excrement, urine, and wound bed. Therefore, examination for urine imposes special significance. The increased number of yeast-like microbes in the urine microscopic examination can be seen as an alert of candida proliferation.

***Aspergillus* spp. and *Mucor* spp.** The ubiquitous fungi seemingly have special preference for burn wounds [24]. Once invasive infections have developed on burn wounds, the patient's situation will become extremely serious. The invasive infection mostly happens among patients with over 30% burn area 9–15 days after the burn. The local lesion presents the following features: once wound sepsis formed, it develops sharply; the hypha can invade the sub-scar tissues and reach the muscular layer after going through the anadesma. The rapid proliferation can be attributed to the fungi's preference for vessels. Besides, the visceral organ can be attacked as well. The detection rate in routine bacteria culture is only 8%. By tissue culture method which is conducive to early diagnosis, 61% of fungoid can be identified.

Due to the difficulty in differentiating between the fungal hyphae and fibrous tissues in conventional H&E stains, special staining method (Gram staining or PAS staining) shall be employed to help the early diagnosis. Additionally, measures like frozen sections of sub-scar tissues and microscopic examination can also contribute to the early diagnosis depending on the examiners' experience.

13.3.4 Anaerobic Bacteria

Although the anaerobic bacteria are not as common as aerobic bacteria, they are frequently found at wound beds around the crissum and perineum among patients suffering from electrical injury or eschar-caused muscle necrosis [25]. The phenomenon can be explained by the decreased potential of oxidation-reduction process caused by ischemia or hypoxia, which helps the anaerobic bacteria to proliferate. Besides, it is also probable that wound is adjacent to crissum and perineum that anaerobic bacteria live as residents. The burn-related anaerobic bacteria include *Clostridium perfringens*, *Bacteroides melaninogenicus*, *Bacteroides fragilis*, and *Peptococcus* spp. For the prevention and treatment of anaerobic bacteria infections, the key point is to remove the necrotic tissues. Among the systematic antibacterial drugs, metronidazole and tinidazole can serve as broad-spectrum drugs against the anaerobic bacteria.

As a kind of obligate anaerobe, *Clostridium tetani* (tetanus) presents positive results in Gram staining. It has a long body like a matchstick, with a round spore on its top-end. It usually lives inside the intestinal tract of humans and animals and can be discharged with the excrement. In the natural environment, it exists ubiquitously in the form of a spore, with special preference for soil and extraordinary resilience against adverse conditions. Given that it is the burn depth rather than burn area that causes tetanus to burn patients, measures against tetanus, such as early hypodermic injection of tetanus antitoxin serum (1500–3000 units), should be applied for patients suffering from severe burns. It should be noted that in some cases, the incubation period is much shorter than the general period of 6–10 days. The function of antitoxin serum is to suppress the free exotoxin, rather than the proliferation of *Clostridium tetani*. Once the free exotoxin has combined with nerve tissues, the antitoxin serum will fail. Since the antitoxin serum's effects can only last for around 10 days, it is recommended that another injection is given to severe burn patients whose necrotic tissues haven't been thoroughly removed 1 week after the first injection. The ultimate approach is to thoroughly remove the necrotic tissues to ease the ischemia or hypoxia environment.

13.3.5 Viral Infection

As a common cause for infections, herpes simplex viruses are mostly found at noses and lips with newly healed superficial second-degree wounds. Other viruses like cytomegalovirus or cowpox virus mostly infect children with superficial burns. In the case of varicella zoster virus (VZV) infections, varicella-like herpes is usually

found on the patients' foreheads or eyelids; the viruses can be identified through direct microscopic examination for the liquid from broken herpes. When presenting no special symptoms, infections caused by herpes simplex viruses or cytomegalovirus can be diagnosed through increased titers in the fixed titration for serum complement. For burn patients with immunosuppression, the abovementioned viruses can invade the gastrointestinal tract, lung, and other viscera.

13.4 Diagnosis of Systemic Burn Infection

13.4.1 Diagnosis of Burn Sepsis

It can be a quasi-diagnosis as burn sepsis in accordance with the following 6 in the first 11 items; a certain diagnosis of burn sepsis can be concluded if 6 in the first 11 items and any in the 12th item are matched [26–28]:

1. Mental excitement, multilingualism, hallucinations, disorientation, or depression.
2. Abdominal distension and weakened or disappeared bowel sounds.
3. Rapid deterioration on the burn wound, manifesting as damp, dull, necrotic spots and deepened wounds.
4. Center temperature >39.0 °C or <36.5 °C.
5. Progressive tachycardia: adult >130 bpm; children >2 SD above age-specific norms (85% age-adjusted max heart rate).
6. Progressive tachypnea: adult >28 bpm not ventilated; children >2 SD above age-specific norms (85% age-adjusted max respiratory rate).
7. Thrombocytopenia (will not apply until 3 days after initial resuscitation): adult $<50 \times 10^9$/L; children <2 SD above age-specific norms.
8. Peripheral blood white blood count $>15 \times 10^9$/L or $< 5 \times 10^9$/L (neutrophil percentage >0.80 or immature granulocyte >0.10); children <2 SD above age-specific norms.
9. Blood procalcitonin >0.5 g/L [29].
10. Blood sodium >155 mmol/L.
11. Blood sugar, >14 mmol/L (no diabetes history).
12. Blood cultures are positive or effective for antibiotic treatment.

13.4.2 Diagnosis of Burn Wound Sepsis

The burn wound sepsis can be seen as diffusion or development of intrusive infection. Its clinical manifestations include severe infections at the burn wounds with rough shapes and necrotic spots at the bleeding points, evident symptoms of systematic infections with negative blood culture, and large amount of bacteria found in the living tissues around the burn wounds with bacteria count over 100,000/g infected tissues. Bacteria are mostly found gathering around the vessels, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* being the most dominant [30].

13.4.3 Diagnosis of Hematogenous Disseminated Mycosis

The development of the disease is less aggravating than that of bacterial systemic infection. It is often caused by long-term use of antibiotics or hypertonic glucose and other nutrients through central venous catheterization [12]. Diagnosis is as follows:

1. More persistent fever. With aggravated or lessened trance and significantly increased heart rate.
2. Often with oral ulcers, dysphagia, and choking. Throat swab culture often presents fungi positive.
3. Shortness or irregular of breath. Patients can burst respiratory tract spasm or respiratory arrest.
4. Often with diarrhea, mucus excretion, and black stool.
5. Elevated blood sodium and blood sugar.
6. Ascended detection rate of fungi in wound, sputum, and feces, positive fungi in urine culture, and the emergence of a large number of fungal hyphae or mycelia under urinal microscopic examination.
7. The higher fungi positive rate of arterial blood culture. Fungi grow slowly in the common blood medium; special culture medium (such as Sabouraud culture medium) should be chosen.
8. Positive fungi in tissue biopsy or culture.

13.5 Prevention and Treatment of Burn Infection

Since the occurrence and development of sepsis are a complex pathophysiological process, the treatment should be comprehensive, including the earliest removal of infection source, rational uses of antibacterial drugs, continuous blood purification, application of glucocorticoid and immunomodulation, and other targeted supportive treatments [31, 32].

13.5.1 Early Remove of Infection Source

Due to the damages on the skin's defensive barriers and the disturbances of blood circulation, the pathogenic microorganisms can proliferate in the necrotic tissues and protein-rich exudates at the burn wounds, causing prevalent wound infection and sepsis. When the systemic conditions allow, the necrotic tissues should be removed at the earliest time, and the wound should be closed. For patients suffering from electrical injury, crush injury, or ring-shaped deep burn, subfascial exploration should be conducted for suspicious parts which may be cut open to reduce the tensions [33]. If the patients produce stench or present aggravated systemic toxemic symptoms, surgery should be carried immediately to utterly remove the necrotic muscles, with special attention for anaerobic infections.

13.5.2 Proper Application of Antibacterial Drugs

To guarantee highly targeted medication and shift from experience-based medication, regular bacteria monitoring should be conducted for patients suffering from severe burns. According to the results of blood culture, sputum culture, and wound culture, antibacterial drugs featuring high sensitivity and low toxicity should be applied. For severe burn with severe shocks, the early antibacterial medication can be decided on the basis of experience, provided that the bacteria haven't been identified. The potent antibiotics can effectively lower the risk of sepsis during the discovery of edema resorption stage and reduce complications in later period. To avoid dysbacteriosis, the application of antibiotics should be consistent with the principle of "early use and early withdrawal" and should be limited to perioperative period [34]. The wound surface should be applied with anti-infection drugs, excluding those for systemic treatment.

Minor Burns In case of minor burns, the antibiotics can be saved if the wound surface drainage is effective. For moderate or severe burns, the timing and duration of antibiotics should be carefully considered according to practical conditions. Patients with third-degree extensive severe burns should be treated with potent antibiotics as soon as possible [35]. When the conditions are stable, the potent antibiotics can be withdrawn. The following use of antibiotics should be confined to perioperative period. Notably, the antibiotics, especially the broad-spectrum antibiotics, are forbidden for long-term usage regardless of practical conditions, to avoid and decrease complications resulting from antibiotics.

Severe Burns Patients with extensive severe burns and severe shocks usually developed enterogenous infection, which aggravates the shock, slows the recovery, and causes visceral organ dysfunction. Therefore, the patients should be applied with antibiotics targeting the enterogenous bacteria. After the shock period, the antibiotics may be withdrawn or continued according to its current conditions.

Perioperative Period Antibiotic Application The antibiotics should be used 30 min before the surgery and continued for 2–3 days after the surgery.

When Presenting Symptoms of Systemic Infections The patients should be applied with antibiotics with high sensitivity to bacteria, low toxicity, and low endotoxin-releasing features, with reference to the bacteria found in the blood culture or wound culture. Empirical antibiotics can be used before pathogenic bacteria are targeted.

When Broad-Spectrum Antibiotics Are Applied The antifungal drug can be used at the same time. For patients with systemic fungal infections, fluconazole, voriconazole, caspofungin, or amphotericin B may be introduced.

13.5.3 Continuous Blood Purification (CPB)

Through ultrafiltration and adsorption, the continuous blood purification can remove or reduce the level of endotoxin and inflammatory mediator and lessen systemic inflammatory responses to improve the organ functions (especially in the case of acute kidney injury or pulmonary edema) and maintain water and electrolyte balance [36]. There are neither clear evidences for the CBP's healing effects on the sepsis nor consensus on the prognosis for sepsis with reference to the CEP. On one hand, it is still to be further confirmed of the CPB on sepsis alone, through multicenter randomized clinical trial [37]. On the other, CPB indeed offers a promising approach to handle the burn sepsis and improve the treatment effects [38].

13.5.4 Application of Early Enteral Feeding

For severe burn patients, the early enteral feeding is proved to be practicable, safe, and effective, imposing significant influences on the prevention and treatment for the enterogenous infections. It contributes to the intestinal resuscitation not only with less intestinal endotoxin translocation, enterogenous infections, and lower level of inflammatory mediator and hormones but also with improved splanchnic blood flow and visceral functional restoration. According to clinical outcomes, plasma endotoxin can be reduced by 30% for adult patients with 50% TBSA burns in early stages after applying the early enteral feeding [39].

13.5.5 Application of Glucocorticoids

Despite its effects of stabilizing lysosomal membrane, reducing cell damages, and maintaining homoeostasis, the glucocorticoid should not be applied as a general adjunctive therapy for shock patients with infection, due to its relations to repeated infections and growth of new infections. It is only applicable to those who still suffer from hypotensive state after being applied to fluid resuscitation and large doses of vasopressors. As the primary therapy, the hydrocortisone shall only be used for short-term treatment (7d), with a maximum dose of 200 mg per day; it is not suitable for long-term use [40].

13.5.6 Immunomodulation

Sepsis can lead to disorders of inflammatory response and immune suppression. The purpose of immunomodulation is a simultaneous treatment with anti-inflammatory and immune stimulation [41]. Combined use of broad-spectrum inflammatory inhibitor and immune enhancer in the treatment of burn sepsis can significantly ameliorate the immune imbalance of patients and reduce the mortality rate of infection [42].

13.5.7 Symptomatic and Supportive Treatment

Treatments include maintenance of hemodynamic stability and respiratory support; control of hyperglycemia with insulin; correction of water, electrolyte, and acid-based balance disorders; and nutritional support, enteral and parenteral nutrition combined with glutamine, arginine, and Omega-3 fatty acid to avoid anemia and hypoproteinemia. Though the maintenance of the body resistance ability, confidence to overcome the disease of patients is improved [43].

13.5.8 Iatrogenic Infection Prevention

1. To avoid catheter-related infection, catheter indwelling time is not more than 7 days in wound-free site and no more than 5 days in the wound site [44].
2. To prevent respiratory tract infection, respiratory tract infection caused by nebulizer and sputum suction tube should be noticed in patients with severe inhalation injury and tracheotomy.
3. To prevent urethral infection, after shock period, urethral catheterization should be used as less as possible. Urethral catheter should be changed at least one time a week if the urethral catheter is necessary.
4. To prevent cross infection, contact with the mattress, sheets, dressings, and equipment should be disinfected, along with strengthened isolation measures especially in the first 2 weeks before the wound granulation barrier fully forms.

13.5.9 Bacteriological Monitoring of Burn Infection

It helps to identify the most effective antibacterial drugs by regularly monitoring on the epidemiological conditions and antibiotic-sensitivity conditions of the burn-related bacteria. Since the wound surface bacteria are not always the same as the ones under the eschars, it is equally necessary to conduct microorganism species identification, bacteria count, and drug-sensitivity test for the sub-eschar tissues, instead of only focusing on wound surface culture and drug-sensitivity test.

During the first 2 weeks after burn, wound surface culture should be conducted regularly. For extensive burn patients, the surface culture should be carried out every 2–3 days. In each process, samples should be collected from every burned area such as the perioral parts, the neck, the body, the perineum, the crissum, and the limbs, in order to oversee the overall distribution of the bacteria. Invasive infection is highly probable when the bacteria count is over 10^5–10^6/g sub-eschar tissues. For systemic infection patients, blood culture, catheter culture, sputum culture, and urine culture should also be conducted to identify the infection source. As for patients suffering from deep burns or with long-term use of large doses of broad-spectrum antibiotics, anaerobic culture and fungus culture should also be carried out to identify the microorganism species causing the infection, rather than only focusing on aerobic bacteria culture.

Since the sample quality is closely related to the quality of culture reports, it is necessary to follow the standardized procedures when collecting the microorganism samples. Considering its large volume, the standardized procedures for sample collection are presented as follows according to the patients' practical conditions.

The Wound and Tissue Samples For some microorganisms, the patients' wound surface can be a sound culture medium where the resident and contaminated bacteria develop well. Therefore, the resident and contaminated microorganisms on the wound bed should be removed with sterile saline before collecting samples. Then, the fester and other exudates at deeper parts of the wound should be collected using sterile cotton and placed inside a culture medium for inspection. For patients whose conditions allow easy tissue-sample collecting, sterile approaches should be adopted to collect samples (0.2–0.4 g) from the junction between the necrotic tissues and normal tissues and place the samples to sterile tubes for inspection. In comparison with samples from wound surface, the tissue samples are less contaminated by bacteria and colonization; the pathogenic bacteria causing local infection are usually found in the necrotic tissues. Therefore, the tissue samples can better reflect the infection conditions on the wound surface.

Blood Culture When conducting blood culture, serious sterilized measures should be introduced for the skin parts where samples will be collected. Besides, the timing, sample amounts, and sample batches should also be carefully considered. The samples should be collected before antibiotics are applied (for patient with long-term usage of antibiotics, the samples should be collected before the next administration or 6–8 h after the antibiotics are used) or 1 h before fever or chill occurs (in cases where large amount of bacteria invade the blood system). During each time, the sampling amount should be 5–10 ml for adults and 1–5 ml for infants and kids. To dilute the bactericidal substance in the blood like antibacterial drugs or antibodies, the ratio of culture medium to blood samples should be 10 to 1. In terms of sample batches, it is recommended that 2–3 bottles of samples should be prepared for aerobic culture and anaerobic culture, respectively, within 24 h; if bottles are not sufficient, at least one bottle should be guaranteed for both, respectively.

Catheter Culture After strict sterilization, the venous catheter being pulled out from the patient's body and 5 cm from the point-end should be cut off by for examination.

Sputum Culture For patients capable of excreting sputum by themselves, after cleaning the mouth to remove the normal bacterial flora, sputum from deep parts of the throat should be collected through deep cough and placed inside the sterile cup for examination. For patients whose air tubes have been cut open or supported by endotracheal tubes, suction catheter should be applied to reach the lung bronchium through the artificial airway for sputum collecting. For patients suffering from severe or tough conditions, immunodepression or anaerobe-caused pulmonary infection, thyrocrico-centesis transtracheal aspiration (TTA), transthoracic lung

aspiration (LA), or protected specimen brush (PSB) and protected bronchoalveolar lavage (PBAL) through bronchial tube or the artificial airway should be applied to collect sputum without oropharyngeal flora contamination to conduct etiological diagnosis of infection.

Urine Culture For patients capable of discharging urine by themselves, after cleaning the reproductive organs in the morning, the midstream urine (the morning urine is preferred) should be collected directly to the sterile containers and immediately delivered for examination; for patients with indwelling catheters, percutaneous catheters may be applied to collect the urine, rather than collecting directly from urine-collecting bags. Bladder puncture conducted from above the pubic bone is the best sample-collecting approach to assess the infection inside the bladder.

13.5.10 Treatment of Infectious Shock (Septic Shock)

Rapid fluid replacement should be applied to achieve resuscitation in 6 h: (1) central venous pressure (CVP) reaches 8–12 mmHg; (2) mean arterial pressure (MAP) no less than 65 mmHg; (3) urine volume (UV) no less than 0.5 ml kg^{-1} h^{-1}; and (4) central venous oxygen saturation no less than 70% or mixed venous oxygen saturation no less than 65% [45]. By increasing the vascular resistance to raise the mean arterial pressure, the norepinephrine is proved to be more effective in handling the hypotension suffered by the infectious shock patients than the dopamine which increases the cardiac index to raise the MAP. Therefore, in the face of infectious shocks, the norepinephrine (2–20 μg kg^{-1} min^{-1}) should be the primary choice. When the norepinephrine fails, the epinephrine or vasopressin can be applied accordingly [46]. Due to its limited effects on vascular resistance and the stimulating effects on the alpha-and beta-adrenergic receptors at the amount of 10 μg kg^{-1} min^{-1}, the dopamine should be avoided on patients with heart rate of over 120 times/min. The epinephrine (1–10 μg kg^{-1} min^{-1}) should be the last choice.

References

1. Peng YZ, Chen J, Yuan ZQ, Li XL, Luo GX, Wu J. Diagnostic criteria and treatment protocol for post-burn sepsis. Crit Care. 2013;17(1):406.
2. Gong YL, Chen J, Liu CJ, Zhang C, Luo XQ, Peng YZ. Comparison of pathogens and antibiotic resistance of burn patients in the burn ICU or in the common burn ward. Burns. 2014;40:402–7.
3. Appelgren P. A prospective study of infections in burn patients. Burns. 2002;28(1):39–46.
4. Peng YZ, Yuan ZQ. Standardized definitions and diagnostic criteria for infection in bum patients. Chin J Burns. 2007;23(6):404–5.
5. Pham TN, Neff MJ, Simmons JM, Gibran NS, Heimbach DM, Klein MB. The clinical pulmonary infection score poorly predicts pneumonia in patients with burns. J Burn Care Res. 2007;28(1):76–9.
6. Zhang YP. Infection of burn opportunistic bacteria and its deep dissemination. Chin J Surg. 1989;27:751.

7. Ma L, Xiao GX. Burn injury and intestinal infection. Acta Acad Med Mil Tert. 1990;12:6.
8. Fang L, Wang F, Sun K, et al. Analysis on the prevalence of central venous catheter-related infection in burn patients and its risk factors. Chin J Burns. 2016;32:243–8.
9. Gong YL, Yang ZC, Yin SP, Liu MX, Zhang C, Luo XQ, Peng YZ. Analysis of the pathogenic characteristics of 162 severely burned patients with bloodstream infection. Chin J Burns. 2016;32(09):529–35.
10. Xiao GX. Clinical significance of quantitative culture of bacteria in burn patients. Med J Chin PLA. 1981;6:319.
11. Deitch EA. Gut-origin sepsis: evolution of a concept. Surgeon. 2012;10:350–6.
12. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med. 2003;29(4):530–8.
13. Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 2001;7:167–202.
14. Goodwin CW. Parenteral nutrition in thermal injuries. In: Rombeau JL, editor. Clinical nutrition: parenteral nutrition. Philadelphia, PA: W. B. Saunders; 1993. p. 566–84.
15. Rotstein OD, Meakins JL. Diagnostic and therapeutic challenges of intraabdominal infections. World J Surg. 1990;14:159–66.
16. Peng YZ, Xiao GX. Intestinal infection and intestinal lymph circulation after severe burn. Chin J Plast Surg. 1996;12(3):483.
17. Peng YZ, Xiao GX, Wang DW, et al. Role of lipid peroxidation in bacterial translocation from the small intestine after thermal injury in rats. J Med Coll PLA. 1990;5(3):271–7.
18. Morehouse JL, Specian RD, Stewart JJ, et al. Translocation of indigenous bacteria from the gastrointestinal tract of mice after oral ricinoleic acid treatment. Gastroenterology. 1986;91:673–82.
19. Peng YZ, Xiao GX. 42 years' experience on the prevention and treatment of systemic infection after severe burn. Chin J Burns. 2001;4(7):93.
20. Greenhalgh DG. Sepsis in the burn patient: a different problem than sepsis in the general population. Burns Trauma. 2017;5:23.
21. Peng YZ, Xiao GX. Early enteral feeding reduced the level of endotoxin in the intestinal lymph of rats with severe scald injury. Chin J Plast Surg. 1998;14(2):83.
22. Yang ZC, Deng LY, Gong YL, Yin SP, Jiang B, Huang GT, Peng YZ, Hu FQ. Inventory building of phages against extensively drug-resistant Acinetobacter baumannii isolated from wounds of patients with severe burn and related characteristic analysis. Chin J Burns. 2016;32(09):517–22.
23. Mousa HA. Correlation between fungi isolated from burn wounds and burn care units. Burns. 1999;25(2):145–7.
24. Xiao GX, et al. Early diagnosis of Aspergillus infection after burns. Acta Acad Med Mil Tert. 1982;4:124.
25. Wang DW, et al. A preliminary study of anaerobic bacterial infection in burn patients. Chin J Surg. 1983;21:399.
26. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644–55.
27. Greenhalgh DG, Saffle JR, Holmes JH, et al. American Burn Association Consensus Conference to define sepsis and infection in burns. J Burn Care Res. 2007;28(6):776–90.
28. Peng YZ. Clinical characteristics and diagnosis of sepsis in pediatric burn patients. Chin J Burns. 2013;29(1):1–3.
29. Wang F, Hu GZ, Chen J, Gong YL, Yuan ZQ, Peng YZ. Prognostic significance of serum procalcitonin in patients with burn sepsis. Chin J Burns. 2014;30(3):223–6.
30. Sun YH. Burn sepsis and multiple organ dysfunction syndrome. Chin J Burns. 2001;17(3):189–90.
31. Xiao GX. The retrospect and the prospect of our country's prevention and treatment of burn injury. Chin J Burns. 2000;9(2):69.

32. Li HM, Zhang JP, Chen J, Song HP, Liu QS, Fan X, Peng YZ, Wu J. Integration of burn treatment and rehabilitation for a child with extremely severe burn. Chin J Burns. 2015;31(2):130–4.
33. Peng YZ. Improving the management of deep partial thickness burn wound. Chin J Burns. 2005;21(1):12–3.
34. Xu WS. Empirical application of antibiotics in burn infection. Chin J Burns. 2002;18(2):71–2.
35. Zheng F, Wang D, Liu N, Shao X, Jin X. Analysis of evaluation indexes for prognosis of severe burn patients with sepsis. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2017;29(4):327–31.
36. Uchino S, Bellomo R, Goldsmith D, Davenport P, et al. Super high flux hemofiltration: a new technique for cytokine removal. Intensive Care Med. 2002;28:651.
37. Ronco C, Tetta C, Mariano F. Interpreting the mechanisms of continuous renal replacement therapy in sepsis: the peak concentration hypothesis. Artif Organs. 2003;27(9):792–801.
38. You B, Zhang YL, Luo GX, et al. Early application of continuous high-volume haemofiltration can reduce sepsis and improve the prognosis of patients with severe burns. Crit Care. 2018;22:173.
39. Sun KD, Dong ZW, Chen J, Liu P, Gong YL, Peng YZ. Effects of early oral administration of mixed enteral nutritional agent on intestinal mucosal barrier of patients with severe burn injury. Chin J Burns. 2015;31(1):25–9.
40. Peng YZ, Yuan ZQ, Xiao GX. Effects of early enteral feeding on the prevention of enterogenous infection in severely burned patients. Burns. 2001;27:145–9.
41. Morgera S, Slowinski T, Melzer C, et al. Renal replacement therapy with high-cutoff hemofilters: impact of convection and diffusion on cytokine clearances and protein status. Am J Kidney Dis. 2004;43(3):444–53.
42. Peng YZ, Yuan ZQ, Li HB. Removal of inflammatory cytokines and endotoxin by veno-venous continuous renal replacement therapy for burned patients with sepsis. Burns. 2005;31:623–8.
43. Orr PA, Case KO, Stevenson JJ. Metabolic response and parenteral nutrition in trauma, sepsis, and burns. J Infus Nurs. 2002;25(1):45–53.
44. Greenfield E. Infectious complications: prevention and strategies for their control. Nurs Clin North Am. 1997;32(2):297–309.
45. Xiao GX. 20 years experience in the treatment of extensive third degree burns. Med J Chin PLA. 1979;4:70.
46. Yao YM, Sheng ZY, Chai JK. The pathogenesis and management of severe sepsis after burns. Chin J Burns. 2008;24(5):337–9.

Modulation of HMGB1 Release for Treating Lethal Infection and Injury

14

Weiqiang Chen, Li He, Hui Jin, John D'Angelo, Guoqiang Bao, and Haichao Wang

Abstract

Sepsis refers to a life-threatening organ dysfunction caused by a dysregulated host response to infection. Its pathogenesis is partly attributable to dysregulated inflammatory responses orchestrated by innate immune cells (e.g., macrophages and monocytes) that sequentially release early (e.g., TNF, IL-1, and IFN-γ) and late (e.g., HMGB1) pro-inflammatory mediators. As a ubiquitous nuclear protein, HMGB1 is constitutively expressed and can be actively secreted in response to exogenous pathogen-associated molecular pattern molecules (PAMPs, e.g., ds-RNA, CpG-DNA, and endotoxin) or endogenous cytokines [e.g., interferon (IFN)-γ or IFN-β]. In addition to active secretion, HMGB1 can also be passively released from damaged cells following ischemia/reperfusion, trauma, or toxemia, thereby serving as damage-associated molecular pattern (DAMP). Even during microbial infections, the PAMP-elicited inflammatory response may be

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accompanied by cell injury and DAMP release that further amplifies the cytokine storm to precipitate organ dysfunction. Here we discuss the evidence that support extracellular HMGB1 as a key mediator of inflammatory diseases and discuss the potential of several HMGB1-targeting therapies in animal models of lethal sepsis. Although microbial infection-induced sepsis is indistinguishable from sterile injury-elicited systemic inflammatory response syndrome, it may be more advantageous to develop strategies that specifically attenuate DAMP-mediated inflammatory responses without compromising the PAMP-mediated innate immunity.

Keywords
Innate immune cells · PAMPs, DAMPs, HMGB1, herbal components · Sepsis · Autophagy · Endocytosis · PKR

14.1 Introduction

The high-mobility group box 1 (HMGB1) is a highly conserved 30 kDa DNA-binding protein constitutively expressed in most types of cells. Bearing two nuclear-localization sequences (NLS), HMGB1 is normally transported into the nucleus, thereby preserving a nuclear "pool" of preformed protein [1–3]. Structurally, it carries two internal repeats of positively charged domains (termed the "HMG boxes" known as "A box" and "B box") in the N-terminus and a continuous stretch of negatively charged acidic residues in the C-terminus (Fig. 14.1). These HMG boxes enable HMGB1 to bind chromosomal DNA to fulfill its nuclear functions in stabilizing nucleosomal structure and regulating gene expression [4]. The conditional disruption of HMGB1 expression in specific tissues uniformly renders animals more susceptible to infectious [5] or injurious insults [6, 7], indicating an important physiological role for the intracellular HMGB1. In response to severe injuries or infections, however, HMGB1 is secreted from activated immune cells or passively released from injured cells. If dysregulated, an excessive extracellular HMGB1 accumulation adversely contributes to the pathogenesis of both injury- and infection-elicited inflammatory diseases. In this chapter, we summarize the molecular mechanisms underlying the regulation of active HMGB1 secretion and highlight several potential HMGB1-targeting therapeutic strategies for lethal sepsis and injury.

14.2 Role of Innate Immune Cells in Lethal Systemic Inflammation

As the first layer of defense, the epithelial barriers effectively prevent the access and/or growth of most pathogens. If they are breached, innate immune cells mount immediate biological responses, termed "inflammation," to confine and remove these pathogens [8]. These inflammatory responses are first initiated by innate

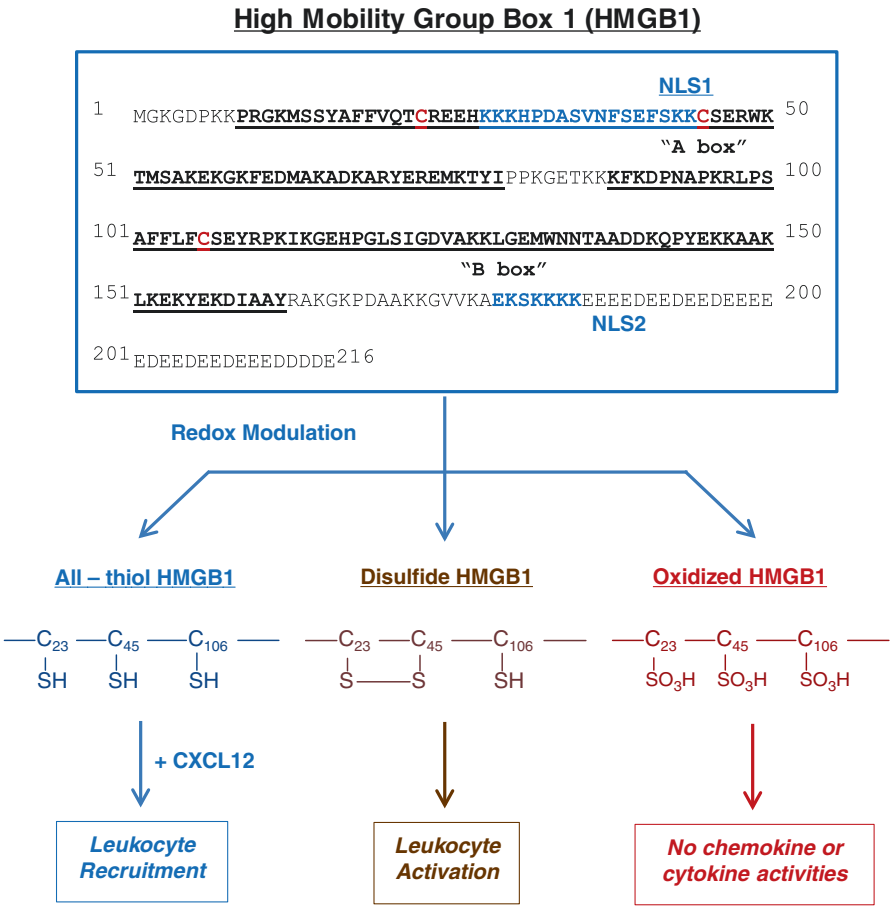

Fig. 14.1 Redox modulates HMGB1 immunological activities. The cysteine residues of HMGB1 can be divergently oxidized, which affects its chemokine or cytokine activities. Depending on the redox status, extracellular HMGB1 can either facilitate leukocyte recruitment or activation, resulting in rigorous inflammatory responses (cytokine storm) and organ dysfunction (adapted from an Open Access Article by [1], An ongoing search for potential targets and therapies for lethal sepsis. *Mil Med Res* 2:20. doi: https://doi.org/10.1186/s40779-015-0047-0)

immune cells such as macrophages and monocytes, which are equipped with various pattern recognition receptors [PRR, such as the Toll-like receptors (TLRs) TLR2, TLR3, TLR4, and TLR9] [9–13] for various pathogen-associated molecular patterns (PAMPs, such as bacterial peptidoglycan, double-stranded RNA, endotoxin, and CpG-DNA, Fig. 14.2) [14, 15]. The engagement of various PAMPs with respective receptors triggers the sequential release of early pro-inflammatory mediators (e.g., TNF, IL-1, and IFN-γ) [16, 17].

If these inflammatory responses are appropriately propagated, it often results in successful elimination of invading pathogens. Otherwise, the invading pathogens can leak into the blood stream, triggering a systemic inflammatory response termed "sepsis."

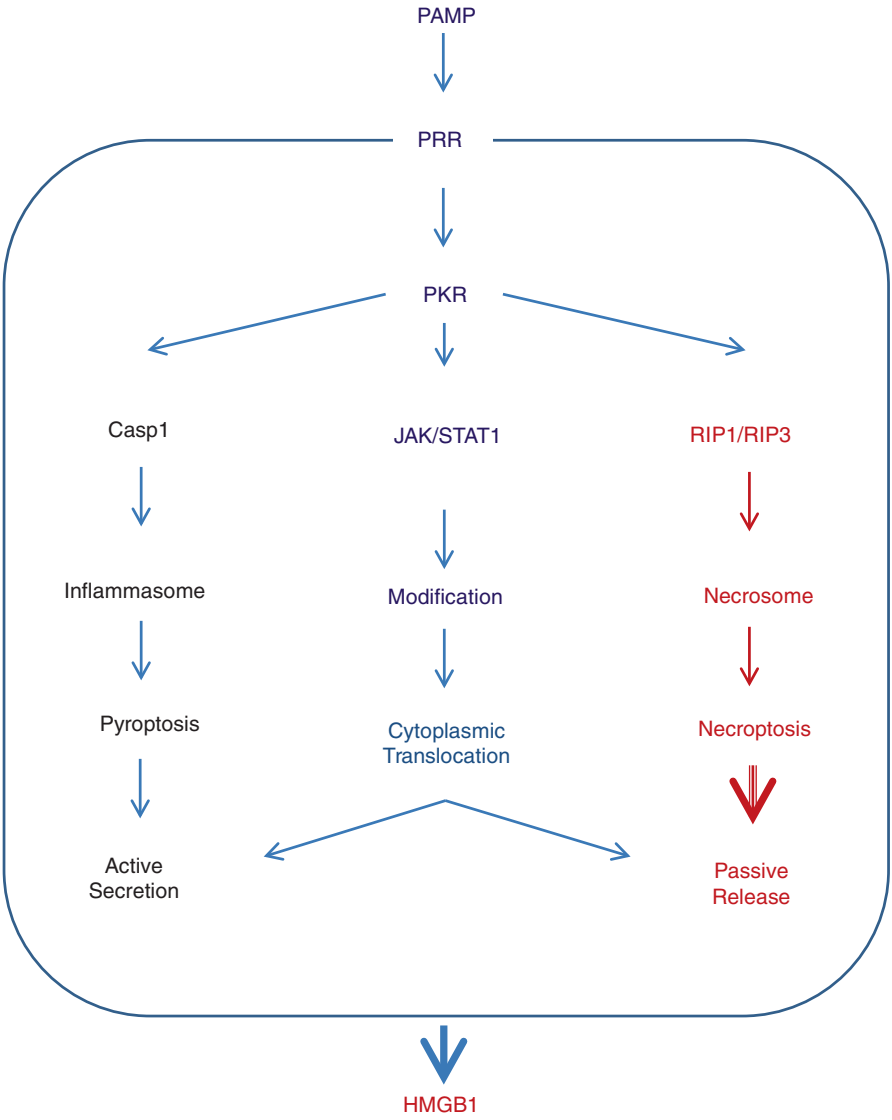

Fig. 14.2 Pathogen-associated molecular patterns (PAMPs) induce active HMGB1 secretion and passive release. Microbial invasion leads to the liberation of PAMPs, which binds to pattern recognition receptors (PRR) to trigger PKR upregulation and phosphorylation. PKR then regulates the JAK/STAT1-dependent nuclear-cytoplasmic HMGB1 translocation, caspase 1-dependent pyroptosis, and/or RIP1-/RIP3-dependent necroptosis. Consequently, HMGB1 is actively secreted or passively released by necrotic cells (adapted from an Open Access Article by [1], An ongoing search for potential targets and therapies for lethal sepsis. *Mil Med Res* 2:20. doi: https://doi.org/10.1186/s40779-015-0047-0)

Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection and represents the leading cause of death in the intensive care unit. As a continuum of increasing clinical severity, "severe sepsis" is often associated with one or more acute organ dysfunctions [18]. Despite recent advances in antibiotic therapy and intensive care, the mortality rate of severe sepsis remains high [19]. Although early cytokines contribute to the pathogenesis of sepsis [20], their early kinetics of production makes them difficult to target in clinical settings. Approximately two decades ago, we initiated an effort to search for other late mediators that could contribute to the pathogenesis of lethal sepsis. Specifically, we stimulated macrophage cultures with an early cytokine TNF and screened the cell-conditioned medium for proteins that were released relatively late. The SDS-PAGE gel electrophoresis revealed the release of 30-kDa protein with an N-terminal amino acid sequence identical to HMGB1 [21], which is a member of the high-mobility group 1 nonhistone chromosomal protein family. Here we describe the molecular mechanisms underlying the regulation of HMGB1 active secretion and passive release, and discuss evidence that support its pathogenic roles in lethal infectious and sterile inflammatory diseases.

14.3 HMGB1's Active Secretion and Passive Release

14.3.1 Active Secretion of HMGB1

In response to exogenous PAMPs (e.g., CpG-DNA, ds-RNA, and endotoxin) [21, 22], or endogenous cytokines [e.g., interferon (IFN)-γ, IFN-β, and cold-inducible RNA-binding protein (CIRP)] [23–25], macrophages/monocytes actively release HMGB1 (Fig. 14.2). Lacking a classical leader peptide sequence, HMGB1 cannot be secreted through the endoplasmic reticulum—Golgi exocytotic pathways [21]. Instead, activated macrophages/monocytes translocate nuclear HMGB1 into cytoplasmic vesicles destined for secreting into the extracellular environment. Recent evidence reveals that the initial nuclear to cytoplasmic translocation of HMGB1 is regulated by the JAK/STAT1-mediated acetylation, whereas the subsequent extracellular release is partly regulated by the double-stranded RNA-activated protein kinase R (PKR)/inflammasome-mediated pyroptosis (Fig. 14.2).

14.3.1.1 Role of JAK/STAT1 in the Regulation of HMGB1 Nuclear-Cytoplasmic Translocation

The nucleus-to-cytoplasm protein shuttling is partly regulated by posttranslational modifications (e.g., acetylation, phosphorylation, methylation) of the HMGB1 nuclear localization or export sequences (NLS or NES) (Fig. 14.1). Thus, intracellular HMGB1 continually shuttles between the nucleus and the cytoplasm, but the equilibrium is tilted toward nuclear accumulation in quiescent cells [26]. In response to microbial PAMPs or host cytokines (e.g., IFNs), innate immune cells acetylate lysine residues 28, 29, 42, 43, 179, 181, and 183 within the NLS sites, leading to the sequestration of HMGB1 into cytoplasmic vesicles (Fig. 14.2) [2, 25–27]. The acetylation is controlled by histone acetylases (HATs) and histone

deacetylases (HDACs) or other enzymes [such as the poly (ADP-ribose) polymerase-1 (PARP-1)] [28], as pharmacological inhibition of HDACs leads to HMGB1 hyperacetylation and nuclear-cytoplasmic translocation [26]. Recently, it has been suggested that the JAK/STAT1 signaling is critically important for the LPS- or IFN-induced HMGB1 hyperacetylation within the NLS sites, thereby regulating HMGB1 nuclear-cytoplasmic translocation [29] (Fig. 14.2). Indeed, pharmacological inhibition or genetic disruption of the JAK/STAT1 signaling uniformly inhibits HMGB1 secretion induced by IFN-β, IFN-γ, or LPS [30, 31].

The phosphorylation of serine residues within the HMGB1 NLS sites may also contribute to the modulation of HMGB1 cytoplasmic translocation [32]. Although the upstream signaling pathway remains poorly elucidated, the calcium/calmodulin-dependent protein kinase (CaMK) IV might be involved in the regulation of the LPS-induced HMGB1 phosphorylation and release [33]. Unlike macrophages/monocytes, quiescent neutrophils carry HMGB1 mainly in the cytoplasm, because the possible methylation of lysine 42 weakens HMGB1/DNA interaction, forcing nuclear HMGB1 to passively diffuse into the cytoplasm [34]. Thus, neutrophils may serve as another important source of extracellular HMGB1 during infection or injury.

14.3.1.2 Role of PKR in the Regulation of HMGB1 Secretion

Following cytoplasmic translocation, HMGB1 is secreted extracellularly through several parallel pathways, including the caspase-1-/caspase-11-mediated inflammasome activation and pyroptosis (Fig. 14.2). Indeed, pharmacological inhibition with a broad-spectrum caspase inhibitor (Z-VAD-FMK), or genetic disruption of caspase-1/caspase-11, uniformly reduces HMGB1 secretion from activated macrophages [35, 36]. Similarly, the genetic disruption of the double-stranded RNA-activated protein kinase R (PKR) or pharmacological suppression of PKR phosphorylation similarly reduces the NLRP3 or NLRP1 agonist-induced inflammasome activation [2, 37], as well as the resultant pyroptosis [29, 37] and HMGB1 release [29]. Notably, ultrapure LPS (*free from contaminating bacterial CpG-DNA or lipoproteins*) failed to trigger PKR phosphorylation and HMGB1 secretion, unless the initial LPS priming is accompanied by a second stimulus, ATP [29, 35]. In contrast, crude LPS (containing bacterial CpG-DNA and lipoproteins) [21], as well as some endogenous pro-inflammatory cytokines, such as the serum amyloid A (SAA) [38] and IFN-γ [39, 40], also effectively upregulated PKR expression and phosphorylation, thereby triggering inflammasome activation and HMGB1 release. In agreement with the two-step control of HMGB1 nuclear translocation and extracellular release, specific inhibition of PKR (with 2-AP) abrogates the LPS-induced HMGB1 release without preventing its nuclear-cytoplasmic translocation. Thus, the LPS-induced HMGB1 cellular secretion is regulated via a two-stage process: (1) the JAK/STAT-mediated nuclear-cytoplasmic translocation and (2) the PKR/inflammasome-dependent pyroptosis and secretion (Fig. 14.2). In light of the possible roles of PKR in the regulation of caspase-1-dependent programmed cell death (pyroptosis) [37] and receptor-interacting protein (RIP)1-/RIP3-dependent programmed necrosis (necroptosis) [40], it is critically important to search for novel PKR inhibitors that may inhibit HMGB1 release by preventing distinct cell death pathways.

14.3.2 Passive Release of HMGB1 from Necrotic Cells

Generally, cell death can be subdivided into two categories: accidental and regulated processes. Accidental cell death can result from mechanical, chemical, or physical destruction of cells and thus does not need specific signaling pathways. Regulated cell death requires the activation of intracellular signaling cascades and can be further subdivided into apoptosis, pyroptosis, and necroptosis, depending on the activation of different caspases or kinases. HMGB1 can be passively released from damaged cells [41] following sterile tissue injury due to ischemia/reperfusion [42, 43], non-penetrating trauma [44, 45], chemical toxemia [46–48], or radiation [49, 50] (Fig. 14.3a). As a DAMP (damage-associated molecular pattern molecule), extracellular HMGB1 alerts innate immune cells to respond to injury [4, 51], triggering an injury-elicited systemic inflammatory response syndrome (SIRS) that resembles microbial infection-induced responses [52]. Some pro-inflammatory cytokines (e.g., TNF and IFN-γ) can also induce a highly regulated programmed necrosis, termed necroptosis (Fig. 14.3b), through activating several signaling molecules such as the protein kinase receptor-interacting protein 3 (RIP3) and PKR. These signaling molecules are involved in the assembly of a "necrosome" protein complex [40, 53, 54], which contributes to passive HMGB1 release (Fig. 14.3). As aforementioned, pyroptosis is a form of regulated cell death that depends on the activation of inflammasomes—protein complexes of a nucleotide-oligomerization domain (NOD)-like receptor (NLR) or the pyrin and HIN domain-containing protein (PYHIN) Aim2, caspase-1, caspase-11, or the adaptor protein. Cells undergoing pyroptosis release cellular content including the pro-inflammatory HMGB1 [55]. Thus, the innate response mechanisms of infection and injury converge on a common process—inflammation [4], which is orchestrated by HMGB1 in conjunction with other pro-inflammatory mediators (e.g., mitochondrial DNA, cold-inducible RNA-binding protein (CIRP)) that are secreted by activated immune cells or released by damaged tissues [24, 56].

14.4 Extracellular HMGB1 as an Alarmin Molecule

Once released, extracellular HMGB1 functions as an alarmin to alert, recruit, and activate immune cells. For example, HMGB1 itself can bind to various PAMPs (e.g., CpG-DNA or LPS), thereby facilitating their recognition by respective receptors [57] and augmenting the PAMP-induced inflammatory responses [57]. Moreover, HMGB1 can enhance the migration of monocytes, dendritic cells [58, 59], and neutrophils [60], functioning as a chemokine to facilitate leukocyte infiltration to the sites of infection or injury [61] (Fig. 14.1). HMGB1 interacts with a family of cell surface receptors and binding proteins including RAGE [57], TLR4 [62], TLR9 [22, 57], cluster of differentiation 24 (CD24)/Siglec-10 [63], Mac-1 [60], thrombomodulin [64], as well as single transmembrane domain proteins (e.g., syndecans) [65]. Consequently, it can activate macrophages [66] and endothelial cells [67] to produce pro-inflammatory cytokines, chemokines, and adhesion molecules (Fig. 14.1).

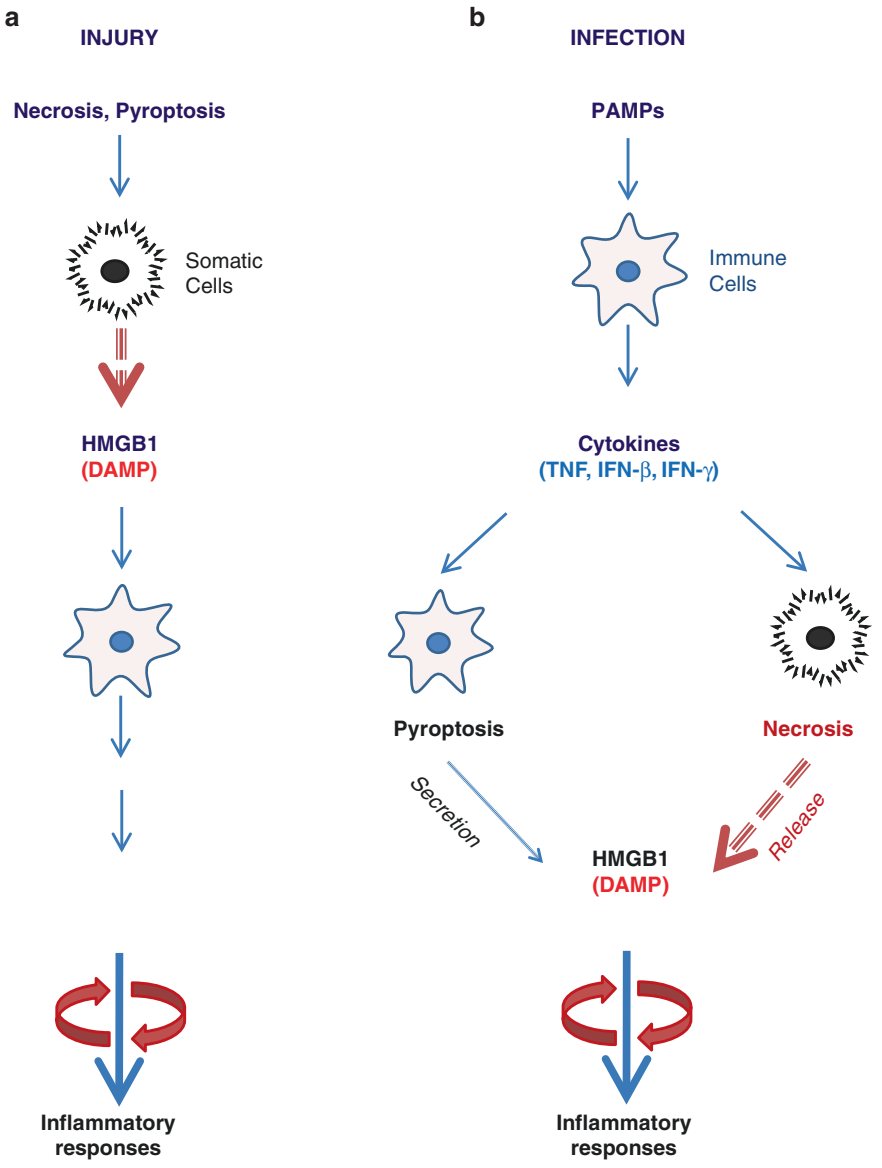

Fig. 14.3 HMGB1 orchestrates injury- and infection-elicited inflammatory responses. (**a**). *Injury triggers passive HMGB1 release*. Following injurious insult, HMGB1 is passively released by necrotic cells and functions as a DAMP signal that propagates rigorous inflammatory responses that are indistinguishable from infection-elicited inflammation. (**b**). *A microbial infection triggers a systemic inflammatory response by stimulating active HMGB1 secretion or passive release.* The disruption of epithelial barrier allows invasion of microbial pathogens, which liberate PAMPs and trigger the production of pro-inflammatory cytokines. Some pro-inflammatory cytokines can stimulate innate immune cells to actively secrete HMGB1 and/or trigger necroptosis that enables passive HMGB1 release. Collectively, extracellular HMGB1 facilitates leukocyte recruitment and activation, amplifying and sustaining rigorous inflammatory responses (adapted from an Open Access Article by [1], An ongoing search for potential targets and therapies for lethal sepsis. *Mil Med Res* 2:20. doi: https://doi.org/10.1186/s40779-015-0047-0)

HMGB1 protein contains three cysteine residues at positions 23, 45, and 106 (C23, C45, and C106, Fig. 14.1), which are sensitive to redox-mediated posttranslational modifications. Specifically, their atomic structures are modified by redox reactions to produce three different HMGB1 isoforms with distinct chemokine- or cytokine-inducing properties (Fig. 14.1) [68–70]. These distinct HMGB1 isoforms are, respectively, termed as to the "all thiol" (fully reduced) form, the "disulfide" (partially oxidized) form, and the "oxidized" form. Specifically, the fully reduced ("all-thiol") HMGB1 binds to chemokines (e.g., CXCL12) and facilitates leukocyte recruitment via the CXCR4 receptor [71]. On the other hand, the partially oxidized HMGB1 can activate immune cells to produce cytokines/chemokines via the TLR4 or other receptors. Once fully oxidized, the HMGB1 is devoid of either chemokine or cytokine activities (Fig. 14.1) [70, 72]. Thus, the redox-mediated modification of HMGB1 serves as a critical regulator of leukocyte recruitment, activation, and subsequent resolution of inflammation.

Notably, different types of inflammasome agonists induce distinct HMGB1 posttranslational modifications. For instance, the NLRP3 inflammasome agonists such as ATP, monosodium uric acids, and adjuvant aluminum induce the secretion of "disulfide" HMGB1 [29]. In contrast, the activation of the NLRC4 inflammasome results in the secretion of fully reduced HMGB1 [55]. One possible reason is that the activation of the NLRP3 inflammasome, but not the NLRC4 inflammasome, is associated with mitochondrial free radical production, which promotes HMGB1 oxidation to form the C23–C45 disulfide bond [73]. Although both the NLRP3 and the NLRC4 inflammasomes similarly mediate the maturation of IL-1β and IL-18, their distinct impact on the redox status of HMGB1 might enable fine-tuning of the immune response against different pathogens.

14.5 HMGB1 as a Mediator of Infection- and Injury-Elicited Inflammation

In response to infection and injury, the host's innate immune system mounts a series of inflammatory response to eliminate the invading pathogens and to heal the wounds [8]. To accomplish this, the innate immune cells (e.g., macrophages/monocytes) are equipped with receptors (e.g., TLR4) that can efficiently recognize both PAMPs (e.g., LPS) [13, 74] and DAMPs (e.g., HMGB1 or CIRP) [24, 75]. The mechanisms underlying the specific recognition of PAMPs and DAMPs might be different, but extensive evidence reveals an essential role for HMGB1 in both infection- and injury-elicited inflammatory diseases.

14.5.1 HMGB1 as a Late Mediator of Sepsis

Substantial evidence has supported the importance to preserve an early PAMP-mediated innate immune response to fight against microbial infection. For example, the impairment of early inflammatory responses often leads to severe immune deficiency during bacterial infection [76]. Although early pro-inflammatory cytokines

(e.g., TNF, IFN-γ) might be protective against microbial infection, the sustained accumulation of late pro-inflammatory mediators (e.g., HMGB1) may adversely contribute to the pathogenesis of lethal infection. In animal models of lethal infection induced by endotoxemia or cecal ligation and puncture (CLP), HMGB1 is first detected in the circulation 8 h after the disease onset and subsequently increased to plateau levels from 16 to 32 h [21, 77]. This late appearance of circulating HMGB1 parallels the onset of animal lethality in models of endotoxemia or sepsis and distinguishes itself from TNF and other early pro-inflammatory cytokines [78].

The pathogenic role of HMGB1 in endotoxemia is inferred from findings that HMGB1-neutralizing antibodies confer a dose-dependent protection against endotoxin-induced animal lethality [21]. In a more clinically relevant animal model of sepsis (induced by CLP), delayed administration of HMGB1-neutralizing antibodies dose-dependently rescue rodents from lethal sepsis [36, 77]. Moreover, targeted inhibition of HMGB1 expression in macrophages and dendritic cells reduces systemic HMGB1 accumulation and confers protection against lethal sepsis [79]. Taken together, these experimental data establish extracellular HMGB1 as a late mediator and therapeutic target of lethal sepsis with a relative wider therapeutic window than other early cytokines.

14.5.2 HMGB1 as an Early Mediator of Traumatic Injury

As a ubiquitous nuclear protein, HMGB1 can also be passively released from necrotic cells [41], thereby serving as a DAMP to elicit inflammatory responses. Following tissue injury, HMGB1 can be passively released from damaged cells. Once reaching the surrounding periphery, HMGB1 amplifies inflammatory responses by inducing various cytokines, chemokines, tissue factor, and adhesion molecules (Fig. 14.3). Following traumatic injury, HMGB1 is detected relatively early in the circulation within a few hours [44, 45, 80], and its systemic levels correlated with dysregulated posttraumatic inflammatory responses [44, 81] and worsening clinical scores [82]. Indeed, substantial evidence has suggested a pathogenic role of HMGB1 in injury (Table 14.1), as HMGB1-neutralizing antibodies are protective in animal models of ischemia/reperfusion [43, 83, 84], trauma [85, 86], chemical toxemia [48, 87, 88], atherosclerosis [89], gastric ulcer [90], and hyperoxia [91].

Notably, it is known that antecedent trauma/tissue damage dampens subsequent innate immune cells to a secondary infection, suggesting a protective role of DAMP in restricting inflammation in response to exogenous pathogen-associated molecular pattern under certain circumstances. As aforementioned, HMGB1 passively released from damaged tissues can bind to cell surface receptor for advanced glycation end products (RAGE) [92], thereby inducing TLR4 internalization and desensitization to subsequent stimulation with microbial ligands (e.g., endotoxin). Thus, although HMGB1 contributes to the pathogenesis of traumatic injury-elicited inflammatory responses, it could also induce tolerance to subsequent infectious inflammation mediated by bacterial endotoxins [93, 94] or lipoteichoic acid (LTA) [95].

Table 14.1 Endogenous HMGB1-inhibiting agents (Adapted from [2], with granted permission from the publisher)

Agents	Infection models	Injury models
Neutralizing antibodies	LPS/CLP	Atherosclerosis Crush Chemical toxemia Liver I/R Brain I/R Heart I/R Hyperoxia Hemorrhagic Trauma Ulcer
Anticoagulant agents Antithrombin III Thrombomodulin	LPS	Ischemia I/R Heatstroke
Acute phase proteins Fetuin-A	LPS/CLP	Cerebral ischemia Burn
Endogenous hormones Vasoactive intestinal peptide	CLP	Ischemia Hemorrhagic injury
Ghrelin	CLP	Intestinal I/R Hypoxia Radiation
Intravenous immunoglobulin	CLP	Cerebral ischemia

Note: *LPS* lipopolysaccharide, *CLP* cecal ligation and puncture, *I/R* ischemia/reperfusion

14.6 Therapeutic Potential of HMGB1-Inhibiting Agents

Our discovery of HMGB1 as a mediator of lethal infection and injury has stimulated the search for endogenous and exogenous agents that can inhibit HMGB1 release to confer protection against inflammatory infection or injury.

14.6.1 Endogenous HMGB1 Inhibitors

Mammals have evolved multiple counter-regulatory anti-inflammatory mechanisms to dampen inflammatory damages to host tissues. For instance, the central nervous system can directly and rapidly downregulate the production of inflammatory mediators via transmitting efferent vagus nerve signals to tissue-resident T cells [96] and macrophages [97]. This anti-inflammatory mechanism is dependent on the release of acetylcholine by specific T cells, as well as the presence of the alpha-7 nicotinic acetylcholine receptor (nAChR) on targeted immune cells [97–99]. At the sites of infection or injury, another biogenic molecule, spermine, is passively released from injured cells and functions as a local counter-regulatory mechanism for PAMP- and DAMP-induced inflammation [100–103]. In addition, the liver strategically re-prioritizes the synthesis and systemic release of a group of proteins collectively termed "acute phase proteins" (APPs), which also serve as counter-regulatory mechanisms

against infection or injury. For instance, the hepatic expression of fetuin-A is negatively regulated by TNF, IL-1, IL-6, and IFN-γ [104] but positively regulated by HMGB1 [104]. In vivo, the supplementation with exogenous fetuin-A confers protection against both injury- [105] and infection-elicited inflammatory responses [104] (Table 14.1). The integral role of fetuin-A in host defense was supported by the observations that fetuin-A-deficient mice were more susceptible to lethal endotoxemic or septic insult [105].

In addition, a number of other endogenous molecules (Table 14.1), including the intravenous immunoglobulin (IVIG) [106], anticoagulant agents (antithrombin III, thrombomodulin) [64, 107], and endogenous hormones (e.g., vasoactive intestinal peptide and ghrelin) [108, 109], have also been proven protective through HMGB1-inhibiting mechanisms. Notably, these endogenous molecules are also protective against sterile ischemia/reperfusion injury [110–114], crush injury [115], burn injury [116], chemical toxemia [117, 118], hypoxic injury [119], and radiation [120] (Table 14.1). It remains elusive whether the protective effects are associated with similar inhibition of HMGB1 release or activities.

14.6.2 Exogenous HMGB1-Inhibiting Agents

A number of herbal extracts (e.g., Danggui, Mung bean, and *Prunella vulgaris*) confer significant protection against lethal endotoxemia or sepsis [121–123]. Similarly, these herbs are also protective against experimental radiation injury [124] and chemical toxemia [125], although it is unknown whether the protection is dependent on the HMGB1 suppression. Currently, an increasing number of herbal components (e.g., nicotine, EGCG, tanshinone, glycyrrhizin, chlorogenic acid, emodin-6-O-β-D-glucoside, rosmarinic acid, isorhamnetin-3-*O*-galactoside, persicarin, forsythoside B, chloroquine, acteroside) (Fig. 14.4) [98, 126–135] have been proven effective in inhibiting endotoxin-induced HMGB1 release.

Interestingly, different herbal components utilize distinct mechanisms to prevent HMGB1 release from activated innate immune cells. For instance, a major green tea component, EGCG, prevents the bacterial endotoxin-induced HMGB1 release by destroying it in the cytoplasm via a cellular degradation process—autophagy [136]. In contrast, a tanshinone IIA derivative, TSN-SS (tanshinone IIA sodium sulfonate), selectively inhibits HMGB1 release by facilitating its endocytosis, leading to subsequent degradation via a lysosome-dependent pathway [137]. A pannexin-1 channel blocker, carbenoxolone (CBX), attenuates endotoxin-induced HMGB1 release by preventing the phosphorylation of PKR [29]. Given the similarity in the chemical structure between CBX and a newly identified PKR inhibitor (7DG), it is important to investigate whether CBX directly binds and inhibits PKR activation.

Given the capacity of herbal ingredients in preventing endotoxin-induced HMGB1 release, we explored their efficacy in animal models of CLP-induced sepsis. Considering the late and prolonged kinetics of HMGB1 accumulation in experimental sepsis [77], the first dose of herbal components was given in a delayed fashion—24 h after the onset of sepsis. Repetitive intraperitoneal administration of

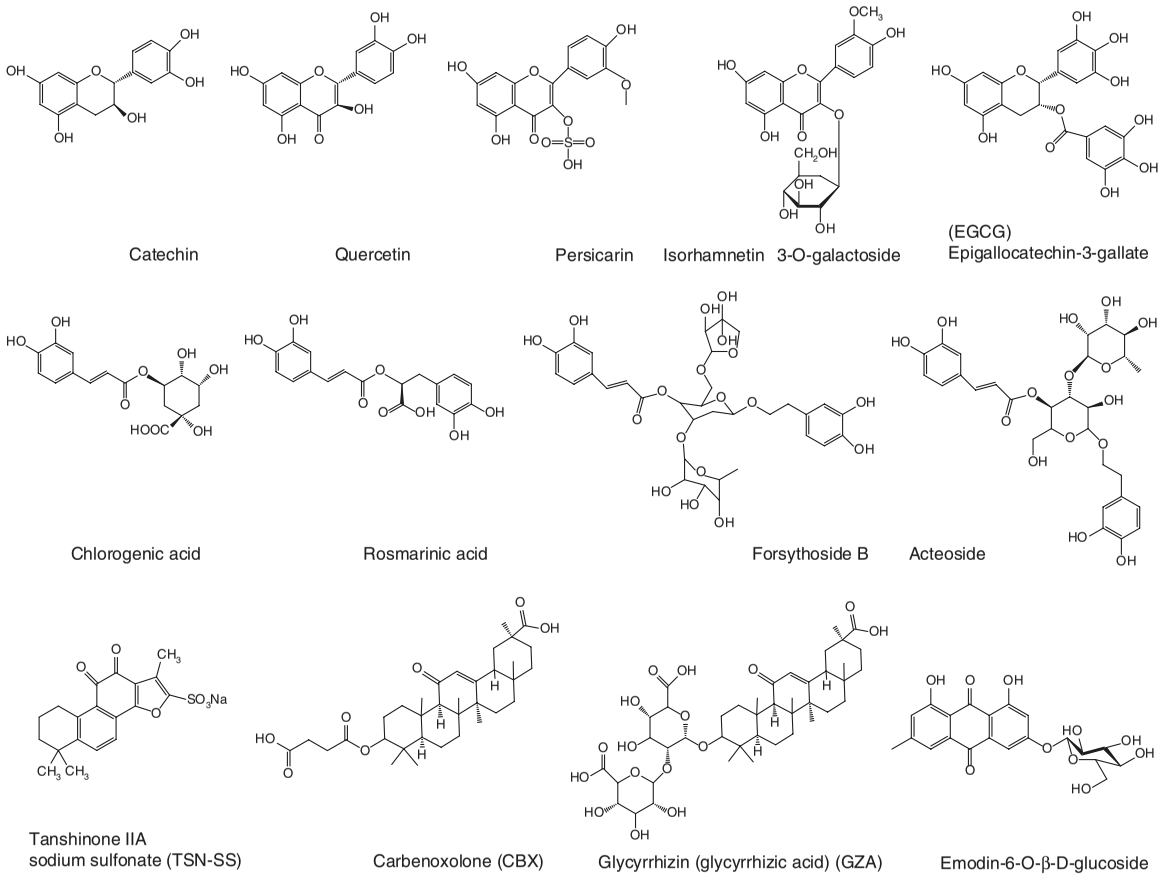

Fig. 14.4 Chemical structures of HMGB1-inhibiting herbal components

EGCG [131], TSN IIA-SS [132], or CBX [138], at 24, 48, and 72 h post CLP, significantly increased animal survival rates. Even when given orally, EGCG also rescued mice from lethal sepsis, significantly increasing animal survival rates from 16% to 44% [136]. Intriguingly, we found that EGCG also facilitated bacterial elimination in the liver and lung of septic animals [139]. It is not yet known whether these antibacterial properties are attributable to EGCG-mediated direct bacteria-killing properties or indirectly through its immune-modulating capacities.

A number of other herbal components have also been proven protective against lethal infection or injury partly by attenuating systemic HMGB1 release or action, stimulating further interest in future clinical studies. Importantly, these herbal components are also protective in animal models of ischemia [140–148], trauma [149, 150], crush injury [151], hemorrhage [152], radiation [153, 154], and chemical toxemia [155, 156] (Table 14.2). Nevertheless, it remains unknown whether the protective effects are associated with their capacity in inhibiting HMGB1 release or chemokine/cytokine activities.

Table 14.2 Exogenous HMGB1-inhibiting agents (Adapted from [2] with granted permission from the publisher)

Agents	Infection models	Injury
Herbal extract		
Danggui	LPS/CLP	Radiation
Mung bean	LPS/CLP	Chemical toxemia
Prunella vulgaris	CLP	–
Herbal components		
Nicotine	LPS/CLP	–
TSN-SS	LPS/CLP	Cerebral ischemia
EGCG	LPS/CLP	Pain Crush injury I/R
Carbenoxolone	CLP	I/R Trauma
Glycyrrhizin	LPS	Vascular injury I/R
Chloroquine	LPS/CLP	I/R Radiation
Acteoside	CLP	Chemical toxemia
Chlorogenic acid	LPS/CLP	Pain I/R
Emodin-6-*O*-β-D-glucoside	CLP	–
Rosmarinic acid (RA)	LPS/CLP	Radiation Oxidative Chemical toxemia
Isorhamnetin-3-*O*-galactoside	LPS/CLP	–
Persicarin	LPS/CLP	I/R
Forsythoside B	CLP	
Higenamine	–	Hypoxic

Notably, agents capable of inhibiting HMGB1 release [98, 131, 132] or action [21, 77] confer protection against sepsis, particularly if given in a delay fashion to strategically preserve the PAMP-mediated early inflammatory response. At a late stage of infection, the PAMP-mediated inflammatory response is likely accompanied by unintended cell injury and DAMP release that further amplifies the cytokine storm to precipitate organ dysfunction [3] (Fig. 14.3b). This possibility is supported by recent findings that HMGB1 is persistently elevated during a late stage of sepsis [157] and contributes to the long-term pathological consequence of sepsis. Although the infection-induced sepsis is similar to the sterile injury-elicited systemic inflammatory response syndrome (SIRS) [52, 158], it may be more effective to develop strategies that specifically attenuate DAMP-mediated inflammatory responses without compromising the PAMP-mediated innate immunity.

14.7 Future Perspectives

Therapeutic strategies targeting PAMPs (e.g., endotoxin) [159] or PAMP signaling (e.g., Eritoran) [160] fail to improve survival in human sepsis clinical trials, raising questions about the feasibility of PAMP-blocking agents in the clinical treatment of infectious diseases. Nevertheless, the investigation of inflammatory cytokines in animal models of diseases has led to the development of successful cytokine-targeting therapies (e.g., chimeric anti-TNF monoclonal antibody, infliximab, and a soluble TNF receptor-Fc fusion protein, sTNF-R-Fc, etanercept) for debilitating chronic inflammatory diseases, such as rheumatoid arthritis (RA) [161]. However, TNF-neutralizing antibodies did not show efficacy in sepsis clinical trials [162]. Thus, it remains highly important to identify other feasible therapeutic targets (such as the anaplastic lymphoma kinase, ALK) for management of inflammatory diseases [163].

In contrast to early cytokines, HMGB1 is secreted from activated innate immune cells and released from damaged cells and functions as an important mediator in lethal infection and injury (Fig. 14.3). In animal model of lethal sepsis, HMGB1-specific neutralizing antibodies or inhibitors can rescue mice from the lethality even if given in a delayed fashion to preserve the potentially beneficial early PAMP-mediated inflammatory responses. It may be important to develop novel strategies to specifically modulate DAMP-elicited excessive inflammation without impairing the PAMP-mediated beneficial innate immunity against infection. Thus, it is important to investigate whether HMGB1 can be a feasible therapeutic target for human sepsis.

Future clinical studies are needed to test the efficacy of HMGB1-neutralizing antibodies in the clinical management of human diseases. However, humanized monoclonal antibodies (mAb) are produced in low-yield mammalian cells and are thus more expensive than small molecule chemical drugs [3]. For example, the dose for frequent injections of Humira (TNF mAb) to treat rheumatoid arthritis is approximately 40 mg every 2 weeks, totaling >1 g (> $16,000) per year. It is thus essential to develop cost-effective small molecule drugs for the human sepsis. One selective

HMGB1 inhibitor, TSN-SS, has been used in China as a therapy for patients with cardiovascular disorders. The dual effects of TSN-SS in inhibiting late inflammatory response and improving cardiovascular function make it a promising drug candidate for sepsis. The capacity to facilitate endocytic HMGB1 uptake by phagocytes may provide basis for treating both infection- and injury-elicited inflammatory diseases [123]. It is thus important to assess whether a better protection could be achieved by combinational therapy using multiple anti-HMGB1 agents.

14.8 Key Issues

1. PAMP stimulates innate immune cells to sequentially release early pro-inflammatory cytokines and late pro-inflammatory mediators that include HMGB1.
2. The secretion of HMGB1 from activated innate immune cells is regulated by JAK/STAT1-mediated cytoplasmic translocation and PKR/inflammasome-dependent pyroptosis and spillage.
3. HMGB1 can be passively released from necrotic cells following ischemia-reperfusion, trauma, and injury, thus serving as a DMAP to orchestrate the injury-elicited inflammatory responses by interacting with a family of receptors.
4. A number of endogenous proteins (e.g., intravenous immunoglobulin, anticoagulants, acute phase proteins, and hormones) are effective in inhibiting HMGB1 release and protecting against lethal infection and injury.
5. Many herbal extracts and components are effective in inhibiting HMGB1 release and protective against lethal infection and injury.
6. Different herbal components (e.g., EGCG, TSN-SS, and CBX) inhibit active secretion of HMGB1 through distinct mechanisms such as via stimulating autophagic HMGB1 degradation, endocytic HMGB1 uptake, or preventing PKR activation.
7. Many agents capable of inhibiting HMGB1 secretion are protective in animal models of injury, but their protective mechanisms remain poorly elucidated.

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References

1. Bao GQ, He L, Lee D, et al. An ongoing search for potential targets and therapies for lethal sepsis. Mil Med Res. 2015;2:20. https://doi.org/10.1186/s40779-015-0047-0. eCollection;%2015.:20-0047.
2. Lu B, Wang C, Wang M, et al. Molecular mechanism and therapeutic modulation of high mobility group box 1 release and action: an updated review. Expert Rev Clin Immunol. 2014;10:713–27.
3. Wang H, Ward MF, Sama AE. Targeting HMGB1 in the treatment of sepsis. Expert Opin Ther Targets. 2014;18:257–68.

4. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. Annu Rev Immunol. 2011;29:139–62. https://doi.org/10.1146/annurev-immunol-030409-101323.
5. Yanai H, Matsuda A, An J, et al. Conditional ablation of HMGB1 in mice reveals its protective function against endotoxemia and bacterial infection. Proc Natl Acad Sci U S A. 2013;110:20699–704.
6. Huang H, Nace GW, McDonald KA, et al. Hepatocyte-specific high-mobility group box 1 deletion worsens the injury in liver ischemia/reperfusion: a role for intracellular high-mobility group box 1 in cellular protection. Hepatology. 2014;59:1984–97.
7. Kang R, Zhang Q, Hou W, et al. Intracellular Hmgb1 inhibits inflammatory nucleosome release and limits acute pancreatitis in mice. Gastroenterology. 2014;146:1097–107.
8. Wang H, Zhu S, Zhou R, Li W, Sama AE. Therapeutic potential of HMGB1-targeting agents in sepsis. Expert Rev Mol Med. 2008;10:e32.
9. Brightbill HD, Libraty DH, Krutzik SR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science. 1999;285:732–6.
10. Gao M, Ha T, Zhang X, et al. Toll-like receptor 3 plays a central role in cardiac dysfunction during polymicrobial sepsis. Crit Care Med. 2012;40(8):2390–9.
11. Ha T, Lu C, Liu L, et al. TLR2 ligands attenuate cardiac dysfunction in polymicrobial sepsis via a phosphoinositide 3-kinase-dependent mechanism. Am J Physiol Heart Circ Physiol. 2010;298:H984–91.
12. Hemmi H, Takeuchi O, Kawai T, et al. A toll-like receptor recognizes bacterial DNA. Nature. 2000;408:740–5.
13. Poltorak A, He X, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science. 1998;282:2085–8.
14. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4:499–511.
15. Baggiolini M, Loetscher P. Chemokines in inflammation and immunity. Immunol Today. 2000;21:418–20.
16. Chan ED, Riches DW. IFN-gamma + LPS induction of iNOS is modulated by ERK, JNK/SAPK, and p38(mapk) in a mouse macrophage cell line. Am J Physiol Cell Physiol. 2001;280:C441–50.
17. Wizemann TM, Gardner CR, Laskin JD, et al. Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. J Leukoc Biol. 1994;56:759–68.
18. Dellinger RP, Levy MM, Carlet JM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med. 2008;36:296–327.
19. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29:1303–10.
20. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature. 1987;330:662–4.
21. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248–51.
22. Ivanov S, Dragoi AM, Wang X, et al. A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA. Blood. 2007;110:1970–81.
23. Kim JH, Kim SJ, Lee IS, et al. Bacterial endotoxin induces the release of high mobility group box 1 via the IFN-beta signaling pathway. J Immunol. 2009;182:2458–66.
24. Qiang X, Yang WL, Wu R, et al. Cold-inducible RNA-binding protein (CIRP) triggers inflammatory responses in hemorrhagic shock and sepsis. Nat Med. 2013;19:1489–95.
25. Rendon-Mitchell B, Ochani M, Li J, et al. IFN-gamma induces high mobility group Box 1 protein release partly through a TNF-dependent mechanism. J Immunol. 2003;170:3890–7.
26. Bonaldi T, Talamo F, Scaffidi P, et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J. 2003;22:5551–60.
27. Gardella S, Andrei C, Ferrera D, et al. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. EMBO Rep. 2002;3:955–1001.

28. Yang Z, Li L, Chen L, et al. PARP-1 mediates LPS-induced HMGB1 release by macrophages through regulation of HMGB1 acetylation. J Immunol. 2014;193:6114–23.
29. Lu B, Nakamura T, Inouye K, et al. Novel role of PKR in inflammasome activation and HMGB1 release. Nature. 2012;488:670–4.
30. Kim YM, Park EJ, Kim JH, et al. Ethyl pyruvate inhibits the acetylation and release of HMGB1 via effects on SIRT1/STAT signaling in LPS-activated RAW264.7 cells and peritoneal macrophages. Int Immunopharmacol. 2016;41:98–105. https://doi.org/10.1016/j.intimp.2016.11.002. Epub;%2016 Nov 16.:98-105.
31. Liu H, Yao YM, Yu Y, et al. Role of Janus kinase/signal transducer and activator of transcription pathway in regulation of expression and inflammation-promoting activity of high mobility group box protein 1 in rat peritoneal macrophages. Shock. 2007;27:55–60.
32. Youn JH, Shin JS. Nucleocytoplasmic shuttling of HMGB1 is regulated by phosphorylation that redirects it toward secretion. J Immunol. 2006;177:7889–97.
33. Zhang X, Wheeler D, Tang Y, et al. Calcium/calmodulin-dependent protein kinase (CaMK) IV mediates nucleocytoplasmic shuttling and release of HMGB1 during lipopolysaccharide stimulation of macrophages. J Immunol. 2008;181:5015–23.
34. Ito I, Fukazawa J, Yoshida M. Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. J Biol Chem. 2007;282:16336–44.
35. Lamkanfi M, Sarkar A, Vande WL, et al. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. J Immunol. 2010;185:4385–92.
36. Qin S, Wang H, Yuan R, et al. Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med. 2006;203:1637–42.
37. Hett EC, Slater LH, Mark KG, et al. Chemical genetics reveals a kinase-independent role for protein kinase R in pyroptosis. Nat Chem Biol. 2013;9:398–405.
38. Li W, Zhu S, Li J, et al. Serum Amyloid A stimulates PKR expression and HMGB1 release possibly through TLR4/RAGE receptors. Mol Med. 2015;21:515–25.
39. Karehed K, Dimberg A, Dahl S, Nilsson K, Oberg F. IFN-gamma-induced upregulation of Fcgamma-receptor-I during activation of monocytic cells requires the PKR and NFkappaB pathways. Mol Immunol. 2007;44:615–24.
40. Thapa RJ, Nogusa S, Chen P, et al. Interferon-induced RIP1/RIP3-mediated necrosis requires PKR and is licensed by FADD and caspases. Proc Natl Acad Sci U S A. 2013;110:E3109–18.
41. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature. 2002;418:191–5.
42. Andrassy M, Volz HC, Igwe JC, et al. High-mobility group box-1 in ischemia-reperfusion injury of the heart. Circulation. 2008;117:3216–26.
43. Tsung A, Sahai R, Tanaka H, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005;201:1135–43.
44. Cohen MJ, Brohi K, Calfee CS, et al. Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. Crit Care. 2009;13:R174.
45. Peltz ED, Moore EE, Eckels PC, et al. HMGB1 is markedly elevated within 6 hours of mechanical trauma in humans. Shock. 2009;32:17–22.
46. Antoine DJ, Dear JW, Lewis PS, et al. Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology. 2013;58:777–87.
47. Seo YS, Kwon JH, Yaqoob U, et al. HMGB1 recruits hepatic stellate cells and liver endothelial cells to sites of ethanol induced parenchymal cell injury. Am J Physiol Gastrointest Liver Physiol. 2013;305(11):G838–48.
48. Zhou RR, Liu HB, Peng JP, et al. High mobility group box chromosomal protein 1 in acute-on-chronic liver failure patients and mice with ConA-induced acute liver injury. Exp Mol Pathol. 2012;93:213–9.
49. Bald T, Quast T, Landsberg J, et al. Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. Nature. 2014;507:109–13.

50. Wang L, He L, Bao G, et al. Ionizing radiation induces HMGB1 cytoplasmic translocation and extracellular release. Guo Ji Fang She Yi Xue He Yi Xue Za Zhi. 2016;40:91–9.
51. Zhu S, Li W, Ward MF, et al. High mobility group box 1 protein as a potential drug target for infection- and injury-elicited inflammation. Inflamm Allergy Drug Targets. 2010;9:60–72.
52. Vincent JL, Opal SM, Marshall JC, Tracey KJ. Sepsis definitions: time for change. Lancet. 2013;381:774–5.
53. Cho YS, Challa S, Moquin D, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell. 2009;137:1112–23.
54. Gunther C, Martini E, Wittkopf N, et al. Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. Nature. 2011;477:335–9.
55. Nystrom S, Antoine DJ, Lundback P, et al. TLR activation regulates damage-associated molecular pattern isoforms released during pyroptosis. EMBO J. 2013;32:86–99.
56. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010;464:104–7.
57. Tian J, Avalos AM, Mao SY, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat Immunol. 2007;8:487–96.
58. Dumitriu IE, Bianchi ME, Bacci M, et al. The secretion of HMGB1 is required for the migration of maturing dendritic cells. J Leukoc Biol. 2007;81:84–91.
59. Yang D, Chen Q, Yang H, et al. High mobility group box-1 protein induces the migration and activation of human dendritic cells and acts as an alarmin. J Leukoc Biol. 2007;81:59–66.
60. Orlova VV, Choi EY, Xie C, et al. A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. EMBO J. 2007;26:1129–11.
61. Degryse B, Bonaldi T, Scaffidi P, et al. The high mobility group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. J Cell Biol. 2001;152:1197–206.
62. Yu M, Wang H, Ding A, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock. 2006;26:174–9.
63. Chen GY, Tang J, Zheng P, Liu Y. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science. 2009;323:1722–5.
64. Abeyama K, Stern DM, Ito Y, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. J Clin Investig. 2005;115:1267–74.
65. Salmivirta M, Rauvala H, Elenius K, Jalkanen M. Neurite growth-promoting protein (amphoterin, p30) binds syndecan. Exp Cell Res. 1992;200:444–51.
66. Zhu S, Ashok M, Li J, et al. Spermine protects mice against lethal sepsis partly by attenuating surrogate inflammatory markers. Mol Med. 2009;15:275–82.
67. Fiuza C, Bustin M, Talwar S, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood. 2003;101:2652–60.
68. Kazama H, Ricci JE, Herndon JM, et al. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. Immunity. 2008;29:21–32.
69. Liu A, Fang H, Dirsch O, Jin H, Dahmen U. Oxidation of HMGB1 causes attenuation of its pro-inflammatory activity and occurs during liver ischemia and reperfusion. PLoS One. 2012;7:e35379.
70. Venereau E, Casalgrandi M, Schiraldi M, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. J Exp Med. 2012;209:1519–28.
71. Schiraldi M, Raucci A, Munoz LM, et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. J Exp Med. 2012;209:551–63.
72. Yang H, Lundback P, Ottosson L, et al. Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). Mol Med. 2012;18:250–9. https://doi.org/10.2119/molmed.2011.00389.

73. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469:221–5.
74. Nagai Y, Akashi S, Nagafuku M, et al. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. Nat Immunol. 2002;3:667–72.
75. Kim S, Kim SY, Pribis JP, et al. Signaling of high mobility group box 1 (HMGB1) through toll-like receptor 4 in macrophages requires CD14. Mol Med. 2013;19:88–98. https://doi.org/10.2119/molmed.2012.00306.
76. Hsu LC, Enzler T, Seita J, et al. IL-1beta-driven neutrophilia preserves antibacterial defense in the absence of the kinase IKKbeta. Nat Immunol. 2011;12:144–50.
77. Yang H, Ochani M, Li J, et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc Natl Acad Sci U S A. 2004;101:296–301.
78. Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. Am J Respir Crit Care Med. 2001;164:1768–73.
79. Ye C, Choi JG, Abraham S, et al. Human macrophage and dendritic cell-specific silencing of high-mobility group protein B1 ameliorates sepsis in a humanized mouse model. Proc Natl Acad Sci U S A. 2012;109:21052–7.
80. Levy RM, Mollen KP, Prince JM, et al. Systemic inflammation and remote organ injury following trauma require HMGB1. Am J Physiol Regul Integr Comp Physiol. 2007;293:R1538–44.
81. Manganelli V, Signore M, Pacini I, et al. Increased HMGB1 expression and release by mononuclear cells following surgical/anesthesia trauma. Crit Care. 2010;14:R197.
82. Sugita A, Kinoshita K, Sakurai A, et al. Systemic impact on secondary brain aggravation due to ischemia/reperfusion injury in post-cardiac arrest syndrome: a prospective observational study using high-mobility group box 1 protein. Crit Care. 2017;21:247–1828.
83. Qiu J, Nishimura M, Wang Y, et al. Early release of HMGB-1 from neurons after the onset of brain ischemia. J Cereb Blood Flow Metab. 2008;28:927–38.
84. Wu H, Ma J, Wang P, et al. HMGB1 contributes to kidney ischemia reperfusion injury. J Am Soc Nephrol. 2010;21:1878–90.
85. Okuma Y, Liu K, Wake H, et al. Anti-high mobility group box-1 antibody therapy for traumatic brain injury. Ann Neurol. 2012;72:373–84.
86. Shimazaki J, Matsumoto N, Ogura H, et al. Systemic involvement of high-mobility group box 1 protein and therapeutic effect of anti-high-mobility group box 1 protein antibody in a rat model of crush injury. Shock. 2012;37:634–8.
87. Nadatani Y, Watanabe T, Tanigawa T, et al. High mobility group box 1 promotes small intestinal damage induced by nonsteroidal anti-inflammatory drugs through toll-like receptor 4. Am J Pathol. 2012;181:98–110.
88. Yang R, Zhang S, Cotoia A, et al. High mobility group B1 impairs hepatocyte regeneration in acetaminophen hepatotoxicity. BMC Gastroenterol. 2012;12:45. https://doi.org/10.1186/1471-230X-12-45.
89. Hirata Y, Kurobe H, Higashida M, et al. HMGB1 plays a critical role in vascular inflammation and lesion formation via toll-like receptor 9. Atherosclerosis. 2013;231:227–33.
90. Nadatani Y, Watanabe T, Tanigawa T, et al. High-mobility group box 1 inhibits gastric ulcer healing through toll-like receptor 4 and receptor for advanced Glycation end products. PLoS One. 2013;8:e80130.
91. Patel VS, Sitapara RA, Gore A, et al. High mobility group Box-1 mediates hyperoxia-induced impairment of Pseudomonas aeruginosa clearance and inflammatory lung injury in mice. Am J Respir Cell Mol Biol. 2013;48:280–7.
92. Li Z, Scott MJ, Fan EK, et al. Tissue damage negatively regulates LPS-induced macrophage necroptosis. Cell Death Differ. 2016;23:1428–47.
93. Aneja RK, Tsung A, Sjodin H, et al. Preconditioning with high mobility group box 1 (HMGB1) induces lipopolysaccharide (LPS) tolerance. J Leukoc Biol. 2008;84:1326–34.
94. El Gazzar M, Yoza BK, Chen X, et al. Chromatin-specific remodeling by HMGB1 and linker histone H1 silence proinflammatory genes during endotoxin tolerance. Mol Cell Biol. 2009;29(7):1959–71.

95. Robert SM, Sjodin H, Fink MP, Aneja RK. Preconditioning with high mobility group box 1 (HMGB1) induces lipoteichoic acid (LTA) tolerance. J Immunother. 2010;33:663–71.
96. Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science. 2011;334:98–101.
97. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405:458–62.
98. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med. 2004;10:1216–21.
99. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature. 2003;421:384–8.
100. Wang H, Zhang M, Bianchi M, et al. Fetuin (alpha2-HS-glycoprotein) opsonizes cationic macrophagedeactivating molecules. Proc Natl Acad Sci U S A. 1998;95:14429–34.
101. Zhang M, Borovikova LV, Wang H, Metz C, Tracey KJ. Spermine inhibition of monocyte activation and inflammation. Mol Med. 1999;5:595–605.
102. Zhang M, Caragine T, Wang H, et al. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. J Exp Med. 1997;185:1759–68.
103. Zhang M, Wang H, Tracey KJ. Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. Crit Care Med. 2000;28:N60–6.
104. Li W, Zhu S, Li J, et al. A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. PLoS One. 2011b;6:e16945.
105. Wang H, Li W, Zhu S, et al. Peripheral administration of fetuin-a attenuates early cerebral ischemic injury in rats. J Cereb Blood Flow Metab. 2010;30:493–504.
106. Hagiwara S, Iwasaka H, Hasegawa A, et al. High-dose intravenous immunoglobulin G improves systemic inflammation in a rat model of CLP-induced sepsis. Intensive Care Med. 2008;34(10):1812.
107. Hagiwara S, Iwasaka H, Matsumoto S, Noguchi T. High dose antithrombin III inhibits HMGB1 and improves endotoxin-induced acute lung injury in rats. Intensive Care Med. 2008;34:361–7.
108. Chorny A, Anderson P, Gonzalez-Rey E, Delgado M. Ghrelin protects against experimental sepsis by inhibiting high-mobility group box 1 release and by killing bacteria. J Immunol. 2008;180:8369–77.
109. Chorny A, Delgado M. Neuropeptides rescue mice from lethal sepsis by down-regulating secretion of the late-acting inflammatory mediator high mobility group box 1. Am J Pathol. 2008;172:1297–307.
110. Fann DY, Lee SY, Manzanero S, et al. Intravenous immunoglobulin suppresses NLRP1 and NLRP3 inflammasome-mediated neuronal death in ischemic stroke. Cell Death Dis. 2013;4:e790. https://doi.org/10.1038/cddis.2013.326.
111. Favreau F, Thuillier R, Cau J, et al. Anti-thrombin therapy during warm ischemia and cold preservation prevents chronic kidney graft fibrosis in a DCD model. Am J Transplant. 2010;10:30–9.
112. Herzog C, Lorenz A, Gillmann HJ, et al. Thrombomodulin's lectin-like domain reduces myocardial damage by interfering with HMGB1-mediated TLR2 signalling. Cardiovasc Res. 2014;101(3):400–10.
113. Jiang W, Tang W, Geng Q, Xu X. Inhibition of toll-like receptor 4 with vasoactive intestinal peptide attenuates liver ischemia-reperfusion injury. Transplant Proc. 2011;43:1462–7.
114. Zhang H, Cui Z, Luo G, et al. Ghrelin attenuates intestinal ischemia/reperfusion injury in mice by activating the mTOR signaling pathway. Int J Mol Med. 2013;32:851–9.
115. Mohri T, Tanaka H, Tajima G, et al. Synergistic effects of recombinant human soluble thrombomodulin and fluid-volume resuscitation in a rat lethal crush injury model. Shock. 2006;26:581–6.
116. Wang XQ, Hayes MT, Kempf M, et al. Fetuin-a: a major fetal serum protein that promotes "wound closure" and scarless healing. J Investig Dermatol. 2008;128:753–7.

117. Imazu Y, Yanagi S, Miyoshi K, et al. Ghrelin ameliorates bleomycin-induced acute lung injury by protecting alveolar epithelial cells and suppressing lung inflammation. Eur J Pharmacol. 2011;672:153–8.
118. Luo Q, Wang Y, Feng D, Xu Y, Xu L. Vasoactive intestinal peptide attenuates concanavalin A-mediated liver injury. Eur J Pharmacol. 2009;607:226–33.
119. Yang D, Liu Z, Zhang H, Luo Q. Ghrelin protects human pulmonary artery endothelial cells against hypoxia-induced injury via PI3-kinase/Akt. Peptides. 2013;42:112–7. https://doi.org/10.1016/j.peptides.2013.01.012.
120. Jacob A, Shah KG, Wu R, Wang P. Ghrelin as a novel therapy for radiation combined injury. Mol Med. 2010;16:137–43.
121. Jun MS, Kim HS, Kim YM, et al. Ethanol extract of Prunella vulgaris var. lilacina inhibits HMGB1 release by induction of heme oxygenase-1 in LPS-activated RAW 264.7 cells and CLP-induced septic mice. Phytother Res. 2012;26:605–12.
122. Wang H, Li W, Li J, et al. The aqueous extract of a popular herbal nutrient supplement, Angelica sinensis, protects mice against lethal endotoxemia and sepsis. J Nutr. 2006;136:360–5.
123. Zhu S, Li W, Li J, et al. It is not just folklore: the aqueous extract of Mung bean coat is protective against sepsis. Evid Based Complement Alternat Med. 2012;2012:498467. https://doi.org/10.1155/2012/498467.
124. Xie CH, Zhang MS, Zhou YF, et al. Chinese medicine Angelica sinensis suppresses radiation-induced expression of TNF-alpha and TGF-beta1 in mice. Oncol Rep. 2006;15:1429–36.
125. Mohd AN, Mohd YH, Long K, et al. Antioxidant and hepatoprotective effect of aqueous extract of germinated and fermented mung bean on ethanol-mediated liver damage. Biomed Res Int. 2013;2013:693613. https://doi.org/10.1155/2013/693613.
126. Jiang WL, Yong X, Zhang SP, Zhu HB, Jian H. Forsythoside B protects against experimental sepsis by modulating inflammatory factors. Phytother Res. 2012;26:981–7.
127. Kim TH, Ku SK, Bae JS. Anti-inflammatory activities of isorhamnetin-3-O-galactoside against HMGB1-induced inflammatory responses in both HUVECs and CLP-induced septic mice. J Cell Biochem. 2013;114:336–45.
128. Kim TH, Ku SK, Bae JS. Persicarin is anti-inflammatory mediator against HMGB1-induced inflammatory responses in HUVECs and in CLP-induced sepsis mice. J Cell Physiol. 2013;228:696–703.
129. Lee CH, Yoon SJ, Lee SM. Chlorogenic acid attenuates high mobility group box 1 (HMGB1) and enhances host defense mechanisms in murine sepsis. Mol Med. 2013;18:1437–48. https://doi.org/10.2119/molmed.2012.00279.
130. Lee W, Ku SK, Kim TH, Bae JS. Emodin-6-O-beta-D-glucoside inhibits HMGB1-induced inflammatory responses in vitro and in vivo. Food Chem Toxicol. 2013;52:97–104. https://doi.org/10.1016/j.fct.2012.10.061.
131. Li W, Ashok M, Li J, et al. A major ingredient of green tea rescues mice from lethal sepsis partly by inhibiting HMGB1. PLoS One. 2007;2:e1153.
132. Li W, Li J, Ashok M, et al. A cardiovascular drug rescues mice from lethal sepsis by selectively attenuating a late-acting proinflammatory mediator, high mobility group box 1. J Immunol. 2007;178:3856–64.
133. Seo ES, Oh BK, Pak JH, et al. Acteoside improves survival in cecal ligation and puncture-induced septic mice via blocking of high mobility group box 1 release. Mol Cells. 2013;35:348–54.
134. Yang EJ, Ku SK, Lee W, et al. Barrier protective effects of rosmarinic acid on HMGB1-induced inflammatory responses in vitro and in vivo. J Cell Physiol. 2013;228:975–82.
135. Yang M, Cao L, Xie M, et al. Chloroquine inhibits HMGB1 inflammatory signaling and protects mice from lethal sepsis. Biochem Pharmacol. 2013;86(3):410–8.
136. Li W, Zhu S, Li J, et al. EGCG stimulates autophagy and reduces cytoplasmic HMGB1 levels in endotoxin-stimulated macrophages. Biochem Pharmacol. 2011;81:1152–63.
137. Zhang Y, Li W, Zhu S, et al. Tanshinone IIA sodium sulfonate facilitates endocytic HMGB1 uptake. Biochem Pharmacol. 2012;84:1492–500.

138. Li W, Li J, Sama AE, Wang H. Carbenoxolone blocks endotoxin-induced protein kinase R (PKR) activation and high mobility group box 1 (HMGB1) release. Mol Med. 2013;19:203–11.
139. Zhao L, Li W, Zhu S, et al. Green tea catechins quench the fluorescence of bacteria-conjugated Alexa fluor dyes. Inflamm Allergy Drug Targets. 2013;12:308–14.
140. Fang H, Liu A, Dahmen U, Dirsch O. Dual role of chloroquine in liver ischemia reperfusion injury: reduction of liver damage in early phase, but aggravation in late phase. Cell Death Dis. 2013;4:e694. https://doi.org/10.1038/cddis.2013.225.
141. Giakoustidis AE, Giakoustidis DE, Iliadis S, et al. Attenuation of intestinal ischemia/reperfusion induced liver and lung injury by intraperitoneal administration of (−)-epigallocatechin-3-gallate. Free Radic Res. 2006;40:103–10.
142. Jiang WL, Tian JW, Fu FH, Zhu HB, Hou J. Neuroprotective efficacy and therapeutic window of Forsythoside B: in a rat model of cerebral ischemia and reperfusion injury. Eur J Pharmacol. 2010;640:75–81.
143. Ogiku M, Kono H, Hara M, Tsuchiya M, Fujii H. Glycyrrhizin prevents liver injury by inhibition of high-mobility group box 1 production by Kupffer cells after ischemia-reperfusion in rats. J Pharmacol Exp Ther. 2011;339:93–8.
144. Tamura K, Alessandri B, Heimann A, Kempski O. The effect of a gap-junction blocker, carbenoxolone, on ischemic brain injury and cortical spreading depression. Neuroscience. 2011;194:262–71.
145. Tsaroucha AK, Valsami G, Kostomitsopoulos N, et al. Silibinin effect on Fas/FasL, HMGB1, and CD45 expressions in a rat model subjected to liver ischemia-reperfusion injury. J Investig Surg. 2017:1–12. https://doi.org/10.1080/08941939.2017.1360416.
146. Wang JG, Bondy SC, Zhou L, et al. Protective effect of Tanshinone IIA against infarct size and increased HMGB1, NFkappaB, GFAP and apoptosis consequent to transient middle cerebral artery occlusion. Neurochem Res. 2014;39:295–304.
147. Yun N, Kang JW, Lee SM. Protective effects of chlorogenic acid against ischemia/reperfusion injury in rat liver: molecular evidence of its antioxidant and anti-inflammatory properties. J Nutr Biochem. 2012;23:1249–55.
148. Zhai CL, Zhang MQ, Zhang Y, et al. Glycyrrhizin protects rat heart against ischemia-reperfusion injury through blockade of HMGB1-dependent phospho-JNK/Bax pathway. Acta Pharmacol Sin. 2012;33:1477–87.
149. Hellmich HL, Rojo DR, Micci MA, et al. Pathway analysis reveals common pro-survival mechanisms of metyrapone and carbenoxolone after traumatic brain injury. PLoS One. 2013;8:e53230.
150. Yin X, Yin Y, Cao FL, et al. Tanshinone IIA attenuates the inflammatory response and apoptosis after traumatic injury of the spinal cord in adult rats. PLoS One. 2012;7:e38381.
151. Renno WM, Al Maghrebi M, Alshammari A, George P. (−)-Epigallocatechin-3-gallate (EGCG) attenuates peripheral nerve degeneration in rat sciatic nerve crush injury. Neurochem Int. 2013;62:221–31.
152. Ohnishi M, Katsuki H, Fukutomi C, et al. HMGB1 inhibitor glycyrrhizin attenuates intracerebral hemorrhage-induced injury in rats. Neuropharmacology. 2011;61:975–80.
153. Lim Y, Hedayati M, Merchant AA, et al. Chloroquine improves survival and hematopoietic recovery after lethal low-dose-rate radiation. Int J Radiat Oncol Biol Phys. 2012;84:800–6.
154. Sanchez-Campillo M, Gabaldon JA, Castillo J, et al. Rosmarinic acid, a photo-protective agent against UV and other ionizing radiations. Food Chem Toxicol. 2009;47:386–92.
155. Kim DW, Cho HI, Kim KM, et al. Isorhamnetin-3-O-galactoside protects against CCl4-induced hepatic injury in mice. Biomol Ther (Seoul). 2012;20:406–12.
156. Lee KJ, Woo ER, Choi CY, et al. Protective effect of acteoside on carbon tetrachloride-induced hepatotoxicity. Life Sci. 2004;74:1051–64.
157. Valdes-Ferrer SI, Rosas-Ballina M, Olofsson PS, et al. High-mobility group box 1 mediates persistent splenocyte priming in sepsis survivors: evidence from a murine model. Shock. 2013;40:492–5.

158. Sursal T, Stearns-Kurosawa DJ, Itagaki K, et al. Plasma bacterial and mitochondrial DNA distinguish bacterial sepsis from sterile systemic inflammatory response syndrome and quantify inflammatory tissue injury in nonhuman primates. Shock. 2013;39:55–62.
159. Ziegler EJ, Fisher CJ Jr, Sprung CL, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A sepsis study group. N Engl J Med. 1991;324:429–36.
160. Opal SM, Laterre PF, Francois B, et al. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. JAMA. 2013;309:1154–62.
161. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu Rev Immunol. 2001;19:163–96.
162. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb sepsis study group. JAMA. 1995;273:934–41.
163. Zeng L, Kang R, Zhu S, et al. ALK is a therapeutic target for lethal sepsis. Sci Transl Med. 2017;9(412):eaan5689.

Sepsis-Induced Lung Injury: The Mechanism and Treatment

15

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Abstract

Sepsis-induced lung injury is one of the severest complications in sepsis. It belongs to the category of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). On the basis of both experimental and clinical studies, the pathogenesis of sepsis-induced lung injury includes the contributions of inflammatory response, vascular hyperpermeability, alveolar edema accumulation, and ventilator-induced lung injury. Survival has been increased with the strategy of protective ventilation based on a ventilator. However, specific effective pharmacologic therapies are still lacking, in spite of the tests of some anti-inflammatory and anticoagulant agents. As new therapeutic strategies, cell therapy and gene therapy are still in their infancy, and their potentials remain to be evaluated. Although a number of basic studies show that some traditional Chinese medicines can protect against ALI or ARDS, it still needs to be further verified by clinical trials.

Keyword

Sepsis-induced lung injury · Acute lung injury · Acute respiratory distress syndrome · Mechanism · Treatment

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15.1 Introduction

Sepsis is an emergency and severe pathologic process with high morbidity and mortality and currently defined as a life-threatening organ dysfunction that resulted from a dysregulated host response to infection [1]. Studies have shown that organ failure is one of the most pernicious complications of sepsis and the lung is the first organ to fail which is known as sepsis-induced lung injury. Semantically, sepsis-induced lung injury belongs to the category of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

The earliest description of ALI/ARDS, however not named in this way, was named as "shock lung" on account of a soldier's exposure to war gas by a doctor serving with the Canadian Forces in 1915. Then, Ashbaugh created the currently acknowledged term ARDS to describe a clinical symptom which was featured by "an acute episodes of tachypnea, hypoxaemia, and loss of compliance after a variety of stimulus" in 1967 [2]. In 1994, the American-European Consensus Conferences (AECC) on ARDS published a declaration on definitions, mechanisms, and cooperation in clinical trials that aimed at guiding the treatment of ALI and ARDS. However, some confusion still existed because of overlapping criteria in regard to the definition of ALI and ARDS [3]. In 2012, according to the degree of hypoxemia, the Berlin definition of ARDS highlighted three categories of ARDS—mild, moderate, and severe. Based on Berlin definition, ALI and ARDS are featured by an acute onset, bilateral infiltration on chest X-ray, pulmonary artery wedge pressure (PAWP) < 18 mmHg, and intractable hypoxemia (PaO_2/FiO_2 ratio < 300 for ALI, PaO_2/FiO_2 ratio < 200 for ARDS) [4]. In 2015, because Riviello et al. worried that the Berlin definition might underrate the incidence of ARDS in low-income countries, they suggested a further revision to the definition which was called the Kigali modification. The Kigali modification tried to recognize ARDS without advanced imaging or testing modalities [5].

ALI/ARDS is common but intractable in the intensive care unit. There are approximately 200,000 new cases each year with a mortality of 41–65% in the United States [6]. There are numerous factors that can cause ALI/ARDS. Whatever can damage alveolar capillary membrane can be called etiological factor of ALI/ARDS. In general, the factors can be classified into direct factors and indirect factors. In detail, the direct factors contain but not limited to inflammation of the lung infection (bacterial, viral, or fungal), suction of gastric contents and drowning, pulmonary contusion, fat embolism, etc. The indirect factors include the injuries by systemic processes like sepsis, multiple injuries, pancreatitis, or adverse reactions to massive blood transfusion [7]. Moreover, the mortality of ALI/ARDS is mainly due to nonpulmonary etiologies, and sepsis accounts for a majority proportion of about 80% of deaths. In summary, sepsis is the foremost pathogenic factor of ALI/ARDS in mankind [8].

The two most common ways used to make a sepsis-induced ALI/ARDS model in the laboratory are lipopolysaccharide (LPS)/endotoxin and fecal peritonitis (FP) [9]. Generally speaking, the model made by LPS/endotoxin in animals is capable of reproducing lots of the characteristics of human sepsis [10]. FP can be replicated in

both large and small experimental animals in multiple ways. The most common way to reproduce FP is cecal ligation and puncture (CLP). The operating steps of CLP include two parts: ligation of the cecum under the ileocecal valve and multiple punctures of the cecum using a small (18 or 20 gauge) hypodermic needle [11].

15.2 Clinical Manifestation

The sepsis-induced ALI/ARDS possesses typical clinical manifestations and pathological features and can be divided into three phases, exudative, proliferative, and fibrotic, in which each has different characteristics.

15.2.1 Exudative Phase

The exudative phase involves the first 7 days after sepsis in terms of time. At this stage, the most important change is the disappearance of the intrinsically tight alveolar barrier to fluid and macromolecules resulted from the injuries of alveolar capillary endothelial cells and type I pneumocytes (alveolar epithelial cells). And the outcome is the accumulation of protein-rich edema fluid in the interstitial and alveolar spaces [12]. Moreover, at this early stage, higher concentrations of cytokines (interleukin-1, interleukin-8, tumor necrosis factor-α, etc.) appeared in the lung tissues. Under the influence of the pro-inflammatory mediators, leukocytes (particularly neutrophils) infiltrate into the lung interstitium and alveolus [13, 14]. Besides, the hyaline membrane is formed by cell fragments, concentrated plasma proteins, and ineffective pulmonary surfactant. Alveolar edema results in reduced ventilation and atelectasis, and most of the dependent lung collapse can significantly reduce lung compliance. Shortness of breath and increased work of breathing can lead to respiratory fatigue and eventually to respiratory failure. Laboratory tests and chest radiographs of ALI/ARDS are usually non-specific, and chest X-ray findings rarely show heart enlargement, pleural effusion, or pulmonary vascular redistribution. Among the differential diagnosis of ALI/ARDS, the most common and most important diseases are cardiogenic pulmonary edema, diffuse pneumonia, and alveolar hemorrhage. In summary, since the non-specificity of the early features of ARDS and ALI, consideration must be taken in identification with other diagnoses [15].

15.2.2 Proliferative Phase

In terms of time, the proliferative phase of sepsis-induced ALI/ARDS usually continues from day 7 to day 21. Most patients are able to recover quickly and are released from mechanical ventilation at this stage. Despite this amelioration, dyspnea, tachypnea, and hypoxemia still exist in many patients. Moreover, progressive lung injury and early changes of pulmonary fibrosis can be suffered by many patients at the proliferative phase [16]. Histologically, signs of improvement at this

stage become apparent, including the initial repair of the lungs, the absorption of alveolar exudates, and the transformation of infiltrating cells in the lungs (neutrophils to lymphocytes) [17, 18]. As part of the repairment, type II lung cells begin to proliferate along the alveolar basement membrane. Studies have shown that new pulmonary surfactants can be synthesized and type I lung cells can be differentiated from these specialized epithelial cells. In addition, as a marker of pulmonary fibrosis, the presence of alveolar type III procollagen peptide is closely related to the prolonged clinical process and increased mortality of sepsis-induced ALI/ARDS [19].

15.2.3 Fibrotic Phase

At 3–4 weeks after the initial lung injury, most patients of sepsis-induced ALI/ARDS are able to restore lung function. However, some patients will still move to the fibrosis stage, where patients may need to use mechanical ventilators and/or supplemental oxygen for life support. The histological features are more obvious, mainly as follows: alveolar edema and inflammatory exudate in the early stage have been transformed into extensive ductal and interstitial fibrosis, and the patient's acinar structure is severely damaged, leading to emphysema [20, 21]. Moreover, progressive vascular occlusion and pulmonary hypertension occur mainly resulted from intimal fibrosis in the pulmonary microcirculation. The main outcomes involve increased risk of pneumothorax, decreased lung compliance, and increased lung dead space [22]. Studies have shown that lung biopsy evidence of pulmonary fibrosis is closely associated with increased mortality at any stage of sepsis-induced ALI/ARDS [23].

15.3 Pathogenesis

The pathogenesis of sepsis-induced ALI/ARDS is complex and associated with a number of factors. Studies have shown that the severity of sepsis-induced ALI/ARDS depends on the degree of pulmonary injury and inflammation, the activation of extrinsic and intrinsic coagulation pathway, extravagant cumulation and activation of leukocytes and platelets into the pulmonary alveolus, and the increased permeability of alveolar endothelial and epithelial barriers [24].

15.3.1 Vascular Hyperpermeability

Integrity of the epithelial-endothelial barrier prevents alveolar edema formation and leukocyte extravasation. Vascular endothelial cadherin (VE-cadherin) is a transmembrane protein with a calcium-dependent adhesion role in blood vessel endothelial cell-to-cell contact [25, 26]. The amount and adhesive function of VE-cadherin mediates paracellular routes of edema formation and leukocyte diapedesis through

a well-coordinated balance between activity of tyrosine kinases and phosphatases. In particular, many pro-inflammatory and permeability-induced factors increase vascular permeability, the most important of which is vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF), or the phosphorylation of VE-cadherin at Tyr685 mediated by src [27, 28]. Additionally, the activation of phosphatase SHP-2 by leukocytes can result in the dephosphorylation of the phosphorylated Tyr731 residue of VE-cadherin. In an animal model of vascular hyperpermeability, LPS or VEGF administration has been associated with dissociation of vascular endothelial protein tyrosine phosphatase from VE-cadherin and increased neutrophil infiltration into the lungs [29]. Moreover, lipid mediators may affect VE-cadherin-mediated vascular permeability. In particular, the sphingolipid metabolite sphingosine-1-phosphate (S1P) binds the G-protein-coupled receptor S1Pr1 and induces VE-cadherin localization at the membrane of endothelial cells [30]. Current researches demonstrated that S1P maintains a barrier-protective tone and S1P receptor agonists may represent pharmacological candidates to enhance vascular integrity. However, given the role of S1P in regulating migration and activity of lymphocytes, caution is warranted before introducing any immune-modulatory agent as putative therapeutic agent. Current studies also demonstrated that osteopontin (OPN) increased vascular permeability by downregulating the expressions of tight junction proteins ZO-1 and claudin-5 [31] and that cytochrome P450 epoxygenase 2J2-epoxyeicosatrienoic acids (CYP2J2-EETs) were crucial for RhoA-dependent regulation of cytoskeletal architecture leading to reversible changes in vascular permeability [32].

15.3.2 Alveolar Edema Accumulation and Clearance

Epithelial cell monolayer stabilizes the alveolar barrier, producing surfactant and ensuring alveolar fluid clearance (AFC). In particular, AFC in patients with ALI/ARDS caused by sepsis is severely impaired and is closely related to higher mortality [33]. Several factors have been shown to be responsible for the lower rate of AFC. Hypoxia and hypercapnia from impaired alveolar gas exchange may decrease the density of Na^+/K^+ ATPase on the basolateral membrane. This mechanism seems to be associated with the higher concentrations of reactive oxygen species (ROS) [34, 35]. In addition, mechanical ventilation with high inspiratory pressures seems to impair cAMP-dependent alveolar fluid clearance by impairing nitric oxide production or by disrupting the cell junction and causing epithelial cell death [36]. In this regard, signaling pathways involving adenosine and its receptors on epithelial cells increase the intracellular levels of cAMP and Na^+-K^+ pump activity, and it may be useful in attenuating lung edema and inflammation [37, 38]. Recently, two key inflammatory mediators in the acute phase of ARDS, transforming growth factor beta (TGF-β) and IL-8, have been shown to impair AFC. Oxidized phospholipids generated by oxidative stress from acid challenge induced lung injury via IL-6 production, independently from the canonical TLR4-MyD88 pathway, suggesting that this high-preserved signaling pathway has a role for infectious risk factors of

sepsis-induced ALI/ARDS [39, 40]. Several experimental models of sepsis-associated ARDS have shown that opposing to ACE, AGII, and AT1 receptor, the angiotensin-converting enzyme 2 (ACE2) mitigates lung injury as demonstrated by reduction in edema [41].

15.3.3 Alveolar Epithelial Cell Death

Although neutrophil apoptosis may limit alveolar damage induced by leukocyte infiltration, epithelial and endothelial cell death severely impairs the barrier function of the alveolar wall [41, 42]. Furthermore, mechanical ventilation, depending on the degree of mechanical stress applied on the cell membrane surface, may cause cell death by apoptosis or necrosis [43, 44]. Mechanical ventilation with low distending pressures at end inspiration resulted in pulmonary apoptosis, while a higher mechanical stretch reduced apoptosis and increased necrosis [45]. In an isolated model of lung injury, mechanical stretch phosphorylates key proteins such as Akt and ERK1-2 that are essential in regulating cell survival and death. In addition, viral infection of human bronchial epithelial cells induces the release of chemokine CXCL8 and macrophage inhibitor factor, which is massively released after cell death by necrosis [46]. In light of these findings, modulation of apoptosis might be a promising strategy to mitigate injury in the lung induced by sepsis.

15.3.4 Inflammatory Responses

Extravagant activation of the pro-inflammatory mediator plays a key role in the pathologic process of sepsis and may lead to serious consequences such as lung injury and lung failure [47]. A variety of humoral mediators can be activated during sepsis-induced lung injury. The complement cascade activated by the classical and alternative pathways can produce large amounts of anaphylatoxins C3a and C5a. Moreover, direct complement fixation of damaged lung tissues can change into the C5-C9 attack compound, leading further injuries [48]. Platelet-activating factor plays a very important role in causing contraction of pulmonary blood vessels and bronchi, systemic vasodilation, enhanced capillary permeability, and activation of macrophages and neutrophils to produce higher concentrations of inflammatory mediators [49]. Tumor necrosis factor α (TNF-α), produced by activatory macrophages, is involved in many of the symptoms of sepsis-induced ALI/ARDS. Interleukin 1 (IL-1), produced by tissue-inherent macrophages, is also significant for the dysregulation of the inflammatory reaction. In addition, chemokines such as IL-8 are effective in inducing and activating neutrophils which resultantly upregulate the expression of adhesion molecules on neutrophils, leading to increased aggregation, adhesion, and damage of vascular endothelial cell. Although the endothelial cell usually produces nitric oxide (NO), the presence of an inflammatory response stimulates the expression of an inducible subtype of NO synthase (iNOS), which is overexpressed and capable of producing toxic NO and oxygen free radicals

[50]. A variety of inflammatory cells such as macrophages, neutrophils, and platelets are main participants of inflammation-induced damage. Studies have shown that in the microcirculation of patients with sepsis, the marginalization of activated neutrophils can often be found. This marginalization can release oxygen free radicals, proteases, and neutrophil extracellular traps (NETs) which are an effective way of capturing and sterilizing pathogens in extracellular spaces primarily through NETosis, further leading to lung damage [51, 52]. In addition, alveolar macrophages can be activated by TLR and NLR signal pathways, and activated macrophages can further recruit macrophages and circulatory neutrophils. Almost all of the primary components required for an inflammatory response can be produced by alveolar macrophages, and the process and duration of the inflammatory response are also closely related to macrophages [53]. Studies have shown that when patients are infected, NF-κB activation of neutrophil is significantly associated with the length of time patients spent on the medical ventilator. Patients who do not increase NF-κB nuclear translocation have obviously shorter time on the medical ventilator than the patients whose NF-κB are activated [54]. It is worth noting that the activation of NF-κB is largely dependent upon Akt and p38, so these two kinases are promising as therapeutic targets for mitigating ALI [55, 56]. Experimental models have shown that the severity of lung injury and inflammation is significantly reduced when NF-κB is inhibited [57]. The magnitude of neutrophil infiltration into the lungs parallels the expression of cell surface expression of chemokine receptor CXCR2, not only on leukocytes but also on epithelial cells, endothelial cells, and fibroblasts. In particular, treatment with specific antibodies that block interaction between CXCR2 and KC-MIP 2 was effective in reducing neutrophil recruitment and tissue damage in mice models of lung injury caused by medical ventilator [54]. The previous studies in our lab have demonstrated that heat shock factor 1 (HSF1) alleviated the sepsis-induced ALI and PMN infiltration in mice by suppressing the surface expression of PSGL-1 and CD11b on PMNs during endotoxemia [58].

15.3.5 Ventilator-Induced Lung Injury

Studies have shown that endotracheal intubation and mechanical ventilation are effective ways to save lives in patients with severe sepsis and ALI/ARDS. The main effects of mechanical ventilation are to ensure sufficient oxygen and reduce the work of the respiratory muscles to allow full rest [59]. Unfortunately, endotracheal intubation and mechanical ventilation also have their drawbacks. More and more experimental and clinical evidence has proven its possibly detrimental outcomes in the past 20 years [60]. One of the most potentially detrimental outcomes is the ventilator-induced lung injury (VILI). Experimental models have demonstrated that recurrent alveolar overexpansion and collapse are two essential processes for VILI [61, 62]. In fact, for patients who have been suffered from sepsis-induced lung injury, VILI is a serious second “hit” to the lungs. In addition, both clinical evidences and experimental researches have shown that extravagant mechanical stress is a major cause of lung injury during mechanical ventilation.

Currently, there are significant advances in protective strategies for the potentially undesirable effects of mechanical ventilation with limiting tidal volume, platform pressure, or both [63, 64].

15.3.6 Coagulation Factors

Endovascular thrombosis plays an important role. It not only prevents microbial invasion, limits infection, and prevents the spread of infection and inflammation but also marks the local inflammatory response [47]. Endovascular fibrin deposition and disseminated intravascular coagulation (DIC) are also essential characteristics of sepsis [65]. IL-6 and other mediators promote endovascular coagulation, which is primarily accomplished through inducing the expression of tissue factors on blood mononuclear cells and vascular endothelial cells [66]. When the tissue factor binds to factor VIIa to form an active compound, factors X and IX are converted to enzymatically activated forms. Activation of exogenous and endogenous coagulation pathways can ultimately produce fibrin [67, 68]. Studies have shown that when the function of the protein C-protein S inhibitory pathway is impaired and the antithrombin and protein C are consumed, coagulation is more likely to occur; and when the plasma level of plasminogen activator inhibitor 1 is elevated, the fiber protein solubilization is inhibited [69].

15.4 Treatment

Sepsis-induced ALI/ARDS is very refractory with a high mortality rate. Once discovered, it must be treated immediately. Effective treatment strategies include rapid control of local infections, provision of hemodynamic support, respiratory support, and timely removal of harmful microorganisms.

15.4.1 General Principles

Currently, the treatment of ALI/ARDS caused by sepsis has made great progress, and its mortality has also been greatly reduced. These progress are largely related to (1) rapid identification of sepsis, (2) timely treatment and removal of infection sources, (3) active prevention of complications, (4) prevention of central venous catheter infection, (5) control of nosocomial infections, and (6) abundant nutrition.

15.4.2 Antimicrobial Strategy

In principle, once the blood sample and other related tissue samples are cultured, antibiotic chemotherapy should be given immediately. The choice of initial antimicrobial agents is primarily based on familiarity of possible pathogens of local

infections at specific sites. Importantly, accumulated empirical antimicrobial therapy can be effective against Gram-positive and Gram-negative bacteria while awaiting culture results. In addition, although many drugs that can neutralize endotoxin in the body have emerged, there is still much controversy about whether endotoxin can be used as a therapeutic intervention target. In a clinical trial with placebo as a control, two endotoxin-specific monoclonal antibodies did not perform well and lacked effective therapeutic effects in patients with sepsis caused by Gram-negative bacteria. Studies have shown that the bactericidal permeability increase (BPI) protein which acts as a human neutrophil protein which neutralizes lipid A can effectively eliminate many Gram-negative bacteria. In addition, a nontoxic lipid A analog that interacts with the TLR4 signaling complex by competing with lipid A can decrease host response to endotoxin [70].

15.4.3 Anti-inflammatory Strategy

Immune activation often occurs when there is local or systemic damage in early septic-induced ALI/ARDS patients. Immune activation is hazardous and can cause pro-inflammatory mediators to be released intrapulmonarily and systemically. However, the treatment of inhibiting pernicious inflammation does not always improve the patient's condition [71]. Targeted specific pro-inflammatory cytokines such as TNF-α and IL-1β have no significant improvement in the prognosis of patients with ARDS and have no effect on the susceptibility of patients with high risk of ARDS [72]. The therapeutic effects of corticosteroids on ALI/ARDS have also attracted widespread attention. In the early (first 48 h) or late (14 days) phases of ALI/ARDS, high doses of corticosteroids did not reduce mortality or effectively treat complications. However, in some respects, the use of more moderate doses of corticosteroids in the early stages of ARDS fibrotic phage may be beneficial for the treatment of the disease, and such studies have been conducted in multicenter phase II/III trials [73, 74]. Other strategies to inhibit the inflammatory process have also failed. Studies have shown that prostaglandin E1, statin drugs, lysophylline, compound ketoconazole, prandial oils, and other antioxidants have no significant effect on ALI/ARDS. Although many studies have demonstrated that neutrophil and/or neutrophil-derived substances are critical for the pathogenesis of ALI/ARDS, and both preclinical and phase II results are encouraging, the use of sivelestat (neutrophil elastase inhibitors) does not ameliorate the medical condition and makes the patient prognosis worse to some extent [75]. The recruitment and activation of neutrophils are dependent on CXC chemokines. Some researchers have proposed the use of CXCR1/CXCR2 (CXC receptor) blockers to inhibit CXC chemokines but have not yet been formally implemented [76]. Current studies have shown that vitamin C and vitamin D3 also play a significant role in fighting inflammation, but the specific mechanism remains unclear, and further research is needed [77, 78].

Regrettably, many drugs with direct or indirect inhibition against inflammatory mediators have not achieved satisfactory results and have not prevented the death of patients with sepsis. The unsuccessful results of these trials may stem from the

effects of many factors. The main factors include inappropriate design of the study (inappropriate endpoints, insufficient sample size, population heterogeneity, numerous covariates) and inappropriate drug administration (inaccurate dose, time or duration of management) [79]. In addition, the use of mAbs in vivo does not effectively neutralize these mediators such as chemokines and cytokines. The main reason is the diversity of these mediators and their overlapping interactions with their receptors.

15.4.4 Management of Mechanical Ventilation

As mentioned earlier, mechanical ventilation (MV) is an effective means of treating ALI/ARDS and can save a patient's life, but at the same time it can also lead to adverse effects which increases lung damage. And studies have shown that alveolar damage resulted from high tidal volume ventilation is one of the most important causes of adverse effects of MV [80]. Based on experimental studies and clinical evidence, many researchers came to the hypothesis that lower tidal volume can prevent lung injury caused by MV and improve the clinical consequences of patients with ALI/ARDS [62, 81]. Moreover, many researchers have verified the hypothesis, and the more authoritative one is sponsored by the National Institutes of Health. In this study, the researchers conducted a large randomized controlled trial of low tidal volume (6 ml/kg) ventilation versus conventional tidal volume (12 ml/kg) and found that patients with low tidal volume had a significant lower mortality rate (31%) than traditional tidal volume patients (40%) [82, 83]. Among all the therapeutic interventions of ALI/ARDS to date, the increase in survival rate due to low tidal volume is the paramount improvement.

In ALI/ARDS, the alveolar and interstitial spaces are filled with protein-rich fluids, and a large amount of surfactant is lost, which can significantly reduce lung compliance. If the end-expiratory pressure does not increase, the alveoli will be severely collapsed, and oxygenation will be impaired. Clinically, based on accumulated experience, positive end-expiratory pressure (PEEP) can be used to minimize FIO2 and maximize PaO2 [59, 84]. The static pressure- volume curve of the respiratory system is requisite for most ventilators today. The lower inflection point (LIP) on the curve represents the alveolar opening and is generally considered as the "best PEEP" for alveolar recruitment with usually 12–15 mmHg. Researchers have attempted to titrate PEEP to a LIP on the hydrostatic-volume curve to improve oxygenation and prevent lung damage [85]. In addition, studies have shown that inverse ratio ventilation (IRV) can increase the average airway pressure to improve oxygenation. IRV mainly means that the inspiratory (I) time is longer than the expiratory (E) time (I: E > 1:1). When the inspiratory time is prolonged and the expiratory time is shortened, dynamic hyperinflation causes an increase in end-expiratory pressure, which is analogical to the PEEP of the ventilator. This IRV mode of ventilation improves oxygenation and has a lower peak pressure than traditional ventilation modes. Regrettably, although IRV has helped a lot in improving oxygenation, it does not reduce the mortality of ALI/ARDS [86]. Present researches have shown

that reduced driving pressure (ΔP = VT/CRS) can increase patient survival and is the optimal ventilation variable to control risk [87]. In addition, MV in the prone position can also improve oxygenation, but it is not clear whether it can improve clinical outcomes. Prone position ventilation has high requirements for the intensive care team and requires a wealth of experience. Otherwise, critically ill patients may have accidental extubation of tracheal intubation and surgical injury. More recently, a multicenter, prospective, randomized, and controlled trial showed that long-term use of the prone position strategy in the early stages of serious ALI/ARDS could significantly reduce 28-day and 90-day mortality rates in patients [88]. Open lung strategy during ARDS aims to decrease the VILI by minimizing the atelectrauma and maldistribution of stress. And current research has proved that the prerequisites of the open lung strategy are not satisfied using PEEP up to 15 cmH_2O and plateau pressure up to 30 cmH_2O. For an effective open lung strategy, higher pressures are required. Therefore, risks of atelectrauma must be weighted versus risks of volutrauma [89].

15.4.4.1 Other Strategies in Mechanical Ventilation

Besides the above ventilation methods, other MV strategies for treating ALI/ARDS such as high frequency ventilation (HFV, 5–20 cycles per second) and low tidal volume (1–2 ml/kg) are also actively tried, but the results are disillusionary [90, 91]. In addition, pulmonary replacement therapy such as extracorporeal membrane oxygenation (ECMO) is a good option for patients who are intractable with traditional ventilation support therapy. ECMO can significantly improve oxygenation and significantly increase CO2 clearance, but rigorous evidence on the optimum timing, disease characteristics, and indications for ECMO in patients with severe ARDS, and its ability to improve short-term and long-term outcomes, must be assessed further before widespread adoption [92, 93]. Moreover, there are many factors affecting the prognosis of patients, including patient age, complications, MV time, and prone position before ECMO. Although complications often occur clinically, the influence on mortality is finite [94]. The employment of ECMO in patients with ALI/ARDS remains controversial, and high-quality research is still needed to further advance our knowledge in the field [95, 96]. Alternatively, lower-flow extracorporeal CO2 removal devices may be used to reduce the intensity of MV (by reducing tidal volume from 6 to 3–4 ml/kg) and to minimize or even abolish the harmful effects of VILI if used as an alternative to conventional MV.

An ongoing study reveals that partial fluid ventilation (PLV) together with perfluorocarbon (an inert high-density fluid that easily dissolves oxygen and carbon dioxide) may promise to restore lung function in patients with ALI/ARDS but still void of patient survival data [97]. Based on current evidence, the approach to mechanical ventilation is recommended.

As mentioned above, although many new strategies in mechanical ventilation are actively studied, effective and complete evidence is still insufficient, which limited their clinical application before more evidence is obtained [98, 99]. Ongoing randomized multicenter clinical trials will test the hypothesis that further lowering of

tidal capacity to 4 ml/kg and plateau pressures to 25 cm H_2O may reduce the risk of VILI and improve survival. Toward this end, partial or total extracorporeal support techniques will be applied to patients with moderate and severe ARDS.

15.4.5 Fluid Management

The most important feature of sepsis-induced ALI/ARDS is protein-rich interstitial and alveolar edema caused by increased pulmonary vascular permeability. Studies have shown that maintaining normal or low left atrial filling pressure can effectively improve pulmonary edema; prevent continuous decline in arterial oxygenation and lung compliance, thereby ameliorate lung dynamics; cut down the hospital stay and MV time; and ultimately decrease mortality rate. Therefore, limiting fluids and using diuretics to reduce left atrial filling pressure is a significant advance in the treatment of ARDS [100].

15.4.6 Anticoagulant Agents

Activated protein C (APC) has been shown to be effective in a randomized controlled trial. For patients with sepsis who experienced the first organ dysfunction, APC or placebo as a control was given within 24 h. The 28-day mortality (24.7%) was significantly lower in the APC group than in the placebo group (30.8%). However, the study also found that the APC group (3%) was more likely to bleed than the placebo group (2%) [101, 102]. Moreover, anti-inflammatory function of APC at therapeutic concentrations may include the inhibition of NETosis formation [103]. But, some studies have proved that whether APC will continue to be used to modulate the acute inflammatory response in humans remains uncertain and there is currently no clear evidence that intravenous injection of rh-APC within 4 days can improve increased alveolar capillary permeability or clinical outcomes in patients with ALI/ARDS [104, 105]. Moreover, heparin derivatives have been approved by FDA, and its effects are worthy of our expectation [66, 106].

15.4.7 Cell-Based Therapies

Currently, a variety of cells, including mesenchymatous stem cells, endothelial progenitor cells, and induced pluripotential stem cells, have been studied for the treatment of ALI/ARDS preclinical models [107]. Bone marrow-derived mesenchymatous stem cells (MSCs) are effective in regulating local and systemic inflammation and are involved in repair responses. The diversity of the function is mainly dependent on the promotion of inherently protective antibacterial effects, the regulation of anti-inflammatory molecules and growth factors, and the differentiation into cells which can replace vascular epithelial cells [108–110]. In a preclinical animal study, exogenous MSCs were shown to significantly reduce the extent of lung injury.

In addition, infusion of cryopreserved human MSCs can significantly decrease the alveolar permeability, inhibit inflammation, and exert antibacterial role after injection of live *E. coli* [111–113]. Encouragingly, these preclinical studies have made a positive impact on Phase I trials, and it has been demonstrated that infusion of allogeneic adeps or bone marrow-derived MSCs is secure and reduces concentrations of circulatory markers resulted from alveolar epithelial damage for moderate to severe ALI/ARDS patients [114, 115]. Moreover, a large-scale polycentric phase II trial of bone marrow-derived human MSC applications is underway, primarily for patients with moderate to severe ARDS [116].

15.4.8 Gene Therapy

Gene therapy can be used to treat sepsis-induced ALI/ARDS, mainly because of its brachychronic but relatively short pathogenesis, consistent with transient gene expression, no need for repeated treatment, and reduced risk of adverse immune responses [117, 118]. A growing body of preclinical evidence suggests that gene therapy can enhance or even restore lung epithelial and/or endothelial cell function, promote lung defense against injury, accelerate inflammation and regression of infection, and improve the prognosis of ALI/ARDS. Recent studies have confirmed that many gene overexpressions, such as angiopoietin-1 [119], HSP-70 [120], apolipoprotein A-1 [121], defensin β2, and the Na^+, K^+-ATPase pump [122], have potential therapeutic effects on ALI/ARDS. Interestingly, the combination of MSCs and EP2 gene therapy has a significant effect on the homing of MSCs to the site of inflammation, which has opened up new avenues for gene therapy in the treatment of inflammatory diseases [123]. Although the current preclinical results are exciting, due to the complexity of the lungs and the high requirements for gene therapy, it is still an arduous task for gene therapy to be truly clinically used for treatment of ALI/ARDS [124].

15.4.9 Traditional Chinese Medicine

With the development of traditional Chinese medicine (TCM) and its effective components, especially their effects on inflammation and oxidative stress, more and more basic research on TCM have been done, but it still needs further verification whether it is effective in clinic. Here, we summarized the roles of TCM in sepsis or sepsis-induced ALI.

15.4.9.1 Huanglian Jiedu Decoction (HJD)

Huanglian Jiedu Decoction (HJD) is a significant traditional Chinese medicine (TCM) which consisted of berberine, astragalus, *Phellodendron amurense* Rupr., and gardenia in a certain proportion. Studies have shown that HJD can inhibit sepsis and inflammation. The main component of *Coptis*, palmatine, is capable of neutralizing LPS with lipid A and reducing the release of pro-inflammatory factors such as

IL-6 and TNF-α. Therefore, HJD is used as an effective attempt to treat sepsis and protect vital organs including the lungs [125].

15.4.9.2 Baicalin

As a flavonoid, baicalin has obvious anti-inflammatory effects and functions to prevent tissue and organ from damage. The use of baicalin can reduce lactate dehydrogenase activity and protein concentration in bronchoalveolar lavage fluid and also reduce lung wet/dry lung weight ratio and pulmonary neutrophil infiltration. In addition, baicalin also significantly inhibited the CX3CL1-CX3CR1 axis and NF-κB pathway in LPS-induced ALI mice. Therefore, baicalin can be used as a potential treatment for ALI/ARDS [126, 127].

15.4.9.3 Baicalein

Baicalein (BE) is a phenolic flavonoid extracted from the roots of *Scutellaria baicalensis* Georgi. Studies have shown that the anti-inflammatory and antioxidative effects of BE are mainly mediated by the inhibition of NF-κB pathway. In addition, it has been demonstrated that BE can also inhibit the upregulation of the Nrf2/HO-1 pathway to protect LPS-induced ALI and ultimately relieve ALI [128, 129].

15.4.9.4 Quercetin

Quercetin is a dietary flavonoid with powerful anti-inflammatory and antioxidant effects. Studies suggest that quercetin can inhibit the secretion of cytokines such as TNF-α, IL-1β, IL-6, NO, and IL-10 in serum induced by LPS and also reduce mortality. In addition, quercetin is also capable of decreasing lung permeability and infiltration of neutrophils and macrophages, also reducing the concentration of MPO and malondialdehyde. Quercetin also significantly inhibited the expression of COX-2, HMGB1, iNOS, and NF-κB p65 phosphorylation. Studies have also shown that quercetin inhibits LPS-induced lung inflammation mainly through the HO-1-dependent pathway. Therefore, quercetin may be an important choice for the treatment of acute lung injury induced by LPS [130–132].

15.4.9.5 Curcumin

The current study has proved that protective effects of intranasal curcumin on LPS-induced lung injury by improvement of the pathological changes in the lungs and decrease in polymorphonuclear leukocyte infiltration, vascular leakage, and pro-inflammatory cytokine release. Therefore, intranasal curcumin might be a therapeutic alternative toward the treatment of ALI/ARDS [133].

15.4.9.6 Escin

Studies have shown that escin has anti-inflammatory and anthydropic effects. Escin is able to inhibit the release of inflammatory mediators including NO, TNF-α, and IL-1β and has a strong protective effect on LPS-induced ALI. Further studies revealed that the upregulation of the glucocorticoid receptor GR and the increase in endogenous antioxidant capacity are the main mechanisms of escin protection [134].

15.4.9.7 Asiaticoside

Asiaticoside (AS) is a triterpene glycoside with strong anti-inflammatory effects and can be isolated from *Centella asiatica*. Studies have shown that the inhibitory effect of AS on LPS-induced inflammatory response of ALI is mainly achieved by the suppression of phosphorylation of NF-κB p65 subunit and the degradation of its inhibitor IκBα. Therefore, AS is likely a potential intervention for ALI/ARDS [135].

15.4.9.8 Salidroside

Salidroside is an important TCM extracted from *Rhodiola*. Researches have demonstrated that pretreatment with salidroside at a dose of 120 mg/kg provides potent protection against LPS-induced ALI with reduced wet/dry weight ratio, MPO activity, protein content, and number of neutrophils and macrophages in bronchoalveolar lavage fluid. In addition, salidroside also inhibits the release of inflammatory cytokines including TNF-α, IL-6, and IL-1β, as well as NF-κB DNA binding activation. In general, salidroside can protect LPS-induced mouse ALI and has certain therapeutic potential [136, 137].

15.4.9.9 Magnolol

Magnolol is a hydroxylated biphenyl compound that inhibits the expression of the iNOS gene and the activation of NF-κB/Rel and p38 kinase, playing a protective effect on LPS-induced ALI. In addition, studies have shown that magnolol can inhibit TLR4-mediated activation of the NF-κB pathway, thereby exerting anti-inflammatory functions. Due to potent anti-inflammatory effects, magnolol may be a potential candidate of treatment strategies for ALI/ARDS in the future [138, 139].

15.4.9.10 Paeoniflorin

As a monoterpene glycoside extracted from the root of *Paeonia lactiflora* Pallas, paeoniflorin (PF) has a protective effect on LPS-induced ALI, which is characterized by attenuating pulmonary edema and reducing inflammatory cell infiltration of lung tissue and inhibiting the release of certain pro-inflammatory cytokines such as IL-1β and TNF-α at transcription and protein levels and reducing microvascular permeability. Furthermore, studies have shown that the protective mechanism of PF on LPS-induced ALI is mainly through inhibition of activation of p38, JNK, and NF-κB pathways in lung tissue. In short, as part of TCM, PF has great potential for the treatment of ALI [140].

15.4.9.11 Resveratrol

As a phytoalexin, resveratrol and its pharmacological activities attract more and more attention. Some studies have shown that resveratrol pretreatment for 3 days has a protective effect on LPS-induced ALI, which is characterized by the inhibition of pulmonary edema, inflammatory cell infiltration, and lung injury. Studies have also shown that the activation of Sirt1 (which is important for inflammation regulation) by resveratrol is also an important reason for protecting LPS-induced ALI. This also reveals that Sirt1 is a potential therapeutic target for the induction of ALI/ARDS in sepsis. In addition, resveratrol can significantly reduce the release of inflammatory

cytokines including IL-1β and MIP-1α and prevent nitric oxide (NO) by inhibiting the expression of inducible NO synthase in lung tissue. Resveratrol pretreatment can also inhibit nuclear translocation of NF-κB in lung tissue. Therefore, the therapeutic potential of resveratrol for ALI/ARDS deserves further study [141–143].

15.4.9.12 Epigallocatechin 3 Gallate (EGCG)

Epigallocatechin gallate (EGCG) is an important TCM. Studies have shown that EGCG can attenuate LPS-induced lung injury, which is mainly dependent on the inhibition of TNF-α release, inhibition of ERK1/2, and JNK activation in macrophages. For ALI/ARDS induced by LPS and seawater inhalation, EGCG pretreatment can inhibit the release of inflammatory cytokines and the activation of JAK/STAT1 pathway, thus realizing its protective effect. In addition, studies have shown that EGCG has potent inhibitive effect against STAT1, which has been shown to be important for inflammatory response. In brief, EGCG has some potential for ALI/ARDS treatment [144, 145].

15.5 Future Directions

How to treat sepsis-induced ALI/ARDS has long been a focus and difficulty for basic researchers and clinicians. Many strategies, including pharmacology and ventilation strategies, are under active research. Since drug development process takes a long time and costs a lot, ventilator-based mechanical ventilation strategy is relatively inexpensive to implement. Indeed, negative fluid balance is currently one of the effective therapies in ALI/ARDS. In addition, low tidal volume ventilation has been widely used clinically in patients with sepsis-induced ALI/ARDS. Many clinical trials against inflammatory factors and cytokines have failed, which suggest that due to the large network of cytokines, treatment aiming at a single cytokine is not feasible and more exploration is required. There are many studies on regulatory pathways, including epigenetic mechanisms that regulate gene expression (such as NF-κB), pathways that regulate necrosis and apoptosis (such as Fas and Fas ligands), and angiogenic factors (such as VEDF and angiopoietin) and renin inhibitors of the angiotensin system. Cell therapy is a novel therapeutic strategy that is still in its infancy, but it has a natural advantage in regulating cell and molecular disorders in ALI/ARDS. Although Chinese medicine is effective in protecting against sepsis-induced ALI or ARDS, more clinical trials need to be done. Considering the diverse causes, complex pathogenesis, and variability of host response in sepsis-induced ALI/ARDS, a specific treatment is unlikely to have same therapeutic effect for all patients, and more effective treatments are still needed.

Future research will test the hypothesis that resting the lung with extracorporeal support will improve lung repair. In addition, based on the most recent advances in mechanisms of lung injury as mentioned before, prevalence and severity of high-permeability lung edema seem to be one of the main clinical features of ARDS. Therefore, there is urgent need for new specific therapies aimed to restore the sealing of the alveolar endothelial barrier and modulate the innate immune response to limit injury and promote resolution.

References

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–10.
2. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet. 1967;2(7511):319–23.
3. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994;149(3):818–24.
4. Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012;307(23):2526–33.
5. Riviello ED, Kiviri W, Twagirumugabe T, et al. Hospital incidence and outcomes of ARDS using the Kigali modification of the Berlin definition. Am J Respir Crit Care Med. 2016;193(1):52–9.
6. Calfee CS, Janz DR, Bernard GR, et al. Distinct molecular phenotypes of direct versus indirect ARDS in single and multicenter studies. Chest. 2015;147(6):1539–48.
7. Tejera P, Meyer NJ, Chen F, et al. Distinct and replicable genetic risk factors for acute respiratory distress syndrome of pulmonary or extrapulmonary origin. J Med Genet. 2012;49(11):671–80.
8. Phua J, Badia JR, Adhikari NK, et al. Has mortality from acute respiratory distress syndrome decreased over time: a systematic review. Am J Respir Crit Care Med. 2009;179(3):220–7.
9. Vincent JL, Abraham E. The last 100 years of sepsis. Am J Respir Crit Care Med. 2006;173(3):256–63.
10. Poli-de-Figueiredo LF, Garrido AG, Nakagawa N, et al. Experimental models of sepsis and their clinical relevance. Shock. 2008;30(1):53–9.
11. Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. Br J Surg. 2001;88(1):22–30.
12. Wittekindt OH. Tight junctions in pulmonary epithelia during lung inflammation. Pflugers Arch. 2017;469(1):135–47.
13. Henderson RB, Hobbs JAR, Mathies M, et al. Rapid recruitment of inflammatory monocytes is independent of neutrophil migration. Blood. 2003;102(1):328–35.
14. Li L, Zhang H, Min D, et al. Sox9 activation is essential for the recovery of lung function after acute lung injury. Cell Physiol Biochem. 2015;37(3):1113–22.
15. Leung WS, Yang ML, Lee SS, et al. Protective effect of zerumbone reduces lipopolysaccharide-induced acute lung injury via antioxidative enzymes and Nrf2/HO-1 pathway. Int Immunopharmacol. 2017;46:194–200.
16. Blondonnet R, Constantin JM, Sapin V, et al. A pathophysiologic approach to biomarkers in acute respiratory distress syndrome. Dis Markers. 2016;2016(21):1–20.
17. Horowitz JC, Cui Z, Moore TA, et al. Constitutive activation of prosurvival signaling in alveolar mesenchymal cells isolated from patients with nonresolving acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol. 2006;290:415–25.
18. Hu R, Cheng Y, Jing H, Wu H, et al. Erythropoietin promotes the protective properties of transplanted endothelial progenitor cells against acute lung injury via PI3K/Akt pathway. Shock. 2014;42(4):327–36.
19. Hu Y, Lou J, Mao YY, et al. Activation of MTOR in pulmonary epithelium promotes LPS-induced acute lung injury. Autophagy. 2016;12(12):2286–99.
20. Gunther A, Mosavi P, Heinemann S. Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia: comparison with the acute respiratory distress syndrome. Am J Respir Crit Care Med. 2000;161:454–62.
21. Day YJ, Chen KH, Chen YL, et al. Preactivated and disaggregated shape-changed platelets protected against acute respiratory distress syndrome complicated by sepsis through inflammation suppression. Shock. 2016;46(5):575–86.
22. Robert SM, Zhu H, Constantin G, et al. Complement inhibition decreases early fibrogenic events in the lung of septic baboons. J Cell Mol Med. 2015;19(11):2549–63.

23. Cross LJM, Matthay MA. Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. Crit Care Clin. 2011;27(2):355–77.
24. Dowdy DW, Eid MP, Dennison CR, et al. Quality of life after acute respiratory distress syndrome: a meta-analysis. Intensive Care Med. 2006;32(8):1115–24.
25. Corada M, Mariotti M, Thurston G, et al. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. Proc Natl Acad Sci. 1999;96:9815–20.
26. Wessel F, Winderlich M, Holm M, et al. Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. Nat Immunol. 2014;15:223–30.
27. Schulte D, Kuppers V, Dartsch N, et al. Stabilizing the VE-cadherin-catenin complex blocks leukocyte extravasation and vascular permeability. EMBO J. 2011;30:4157–70.
28. Sidibe A, Imhof BA. VE-cadherin phosphorylation decides: vascular permeability or diapedesis. Nat Immunol. 2014;15:215–7.
29. Broermann A, Winderlich M, Block H, et al. Dissociation of VE-PTP from VE-cadherin is required for leukocyte extravasation and for VEGF induced vascular permeability in vivo. J Exp Med. 2011;208:2393–401.
30. Camerer E, Regard JB, Cornelissen I, et al. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. J Clin Invest. 2009;119:1871–9.
31. Zhang J, Yang G, Zhu Y, et al. Relationship of Cx43 regulation of vascular permeability to osteopontin-tight junction protein pathway after sepsis in rats. Am J Physiol Regul Integr Comp Physiol. 2017;443:R1.
32. Dong R, Hu D, Yang Y, et al. EETs reduces LPS-induced hyperpermeability by targeting GRP78 mediated Src activation and subsequent Rho/ROCK signaling pathway. Oncotarget. 2017;8(31):50958–71.
33. Peters DM, Vadasz I, Wujak L, et al. TGF-β directs trafficking of the epithelial sodium channel ENaC which has implications for ion and fluid transport in acute lung injury. Proc Natl Acad Sci U S A. 2014;111:E374–83.
34. Roux J, McNicholas CM, Carles M, et al. IL-8 inhibits cAMP-stimulated alveolar epithelial fluid transport via a GRK2/PI3K dependent mechanism. FASEB J. 2013;27:1095–106.
35. Cui Y, Ding Y, Chen L, et al. Dexmedetomidine enhances human lung fluid clearance through improving alveolar sodium transport. Fundam Clin Pharmacol. 2017;31(4):429–37.
36. Frank JA, Pittet JF, Lee H, et al. High tidal volume ventilation induces NOS2 and impairs cAMP- dependent air space fluid clearance. Am J Physiol Lung Cell Mol Physiol. 2003;284:L791–8.
37. Eckle T, Grenz A, Laucher S, et al. A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. J Clin Invest. 2008;118:3301–15.
38. Vadasz I, Sznajder J. Gas exchange disturbances regulate alveolar fluid clearance during acute lung injury. Front Cell Infect Microbiol. 2017;8:757–64.
39. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell. 2008;133:235–49.
40. Mazzocchi LC, Vohwinkel CU, Mayer K, et al. TGF-β inhibits alveolar protein transport by promoting shedding, regulated intramembrane proteolysis and transcriptional downregulation of megalin. Am J Physiol Lung Cell Mol Physiol. 2017;313(5):L807–24.
41. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005;11:875–9.
42. Matthay MA. Resolution of pulmonary edema. Thirty years of progress. Am J Respir Crit Care Med. 2014;189(11):1301–8.
43. Fanelli V, Mascia L, Puntorieri V, et al. Pulmonary atelectasis during low stretch ventilation: "open lung" versus "lung rest" strategy. Crit Care Med. 2009;37:1046–53.
44. Dolinay T, Himes B, Shumyatcher M, et al. Integrated stress response mediates epithelial injury in mechanical ventilation. Am J Respir Cell Mol Biol. 2017;57(2):193–203.

45. Imai Y, Parodo J, Kajikawa O, et al. Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. JAMA. 2003;289:2104–12.
46. Arndt U, Wennemuth G, Barth P, et al. Release of macrophage migration inhibitory factor and CXCL8/interleukin-8 from lung epithelial cells rendered necrotic by influenza A virus infection. J Virol. 2002;76:9298–306.
47. Chimenti L, Camprubí-Rimblas M, Guillamat-Prats R, et al. Nebulized heparin attenuates pulmonary coagulopathy and inflammation through alveolar macrophages in a rat model of acute lung injury. Thromb Haemost. 2017;117(11):2125–34.
48. Bosmann M, Ward PA. Role of C3, C5 and anaphylatoxin receptors in acute lung injury and in sepsis. Adv Exp Med Biol. 2012;946:147–59.
49. Katz JN, Kolappa KP, Becker RC. Beyond thrombosis: the versatile platelet in critical illness. Chest. 2011;139(3):658–68.
50. Williams AE, Chambers RC. The mercurial nature of neutrophils: still an enigma in ARDS? Am J Physiol Lung Cell Mol Physiol. 2014;306(3):L217–30.
51. Rubenfeld GD. Confronting the frustrations of negative clinical trials in acute respiratory distress syndrome. Ann Am Thorac Soc. 2015;12(1):S58–63.
52. McDonald B, Urrutia R, Yipp BG, et al. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. Cell Host Microbe. 2012;12(3):324–33.
53. Leissinger M, Kulkarni R, Zemans RL, et al. Investigating the role of nucleotide-binding oligomerization domain-like receptors in bacterial lung infection. Am J Respir Crit Care Med. 2014;189(12):1461–8.
54. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol. 2010;11(5):373–84.
55. Ray NB, Durairaj L, Chen BB, et al. Dynamic regulation of cardiolipin by the lipid pump Atp8b1 determines the severity of lung injury in experimental pneumonia. Nat Med. 2010;16(10):1120–7.
56. Qi D, Wang D, Zhang C, et al. Vaspin protects against LPS-induced ARDS by inhibiting inflammation, apoptosis and reactive oxygen species generation in pulmonary endothelial cells via the Akt/GSK-3β pathway. Int J Mol Med. 2017;40:1803–17.
57. Simmons JD, Lee YL, Mulekar S, et al. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. Ann Surg. 2013;258(4):591–6.
58. Chen S, Zuo X, Yang M, et al. Severe multiple organ injury in HSF1 knockout mice induced by lipopolysaccharide is associated with an increase in neutrophil infiltration and surface expression of adhesion molecules. J Leukoc Biol. 2012;92(4):851–7.
59. Vieillard-Baron A, Matthay M, Teboul JL, et al. Experts' opinion on management of hemodynamics in ARDS patients: focus on the effects of mechanical ventilation. Intensive Care Med. 2016;42(5):739–49.
60. Chiumello D, Brochard L, Marini JJ, et al. Respiratory support in patients with acute respiratory distress syndrome: an expert opinion. Crit Care. 2017;21(1):240–8.
61. Lista G, Castoldi F, Fontana P, et al. Lung inflammation in preterm infants with respiratory distress syndrome: effects of ventilation with different tidal volumes. Pediatr Pulmonol. 2006;41(4):357–63.
62. Sahetya SK, Mancebo J, Brower RG. 50 years of research in ARDS. Tidal volume selection in the acute respiratory distress syndrome. Am J Respir Crit Care Med. 2017;08:1629–50.
63. Frank JA, Parsons PE, Matthay MA. Pathogenetic significance of biological markers of ventilator-associated lung injury in experimental and clinical studies. Chest. 2006;130(6):1906–14.
64. Goligher EC, Kavanagh BP, Rubenfeld GD, et al. Oxygenation response to positive end-expiratory pressure predicts mortality in acute respiratory distress syndrome. A secondary analysis of the LOVS and ExPress trials. Am J Respir Crit Care Med. 2014;190(1):70–6.
65. Ogura H, Gando S, Iba T, et al. SIRS-associated coagulopathy and organ dysfunction in critically ill patients with thrombocytopenia. Shock. 2007;28(4):411–7.

66. Dixon B, Schultz MJ, Smith R, et al. Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial. Crit Care. 2010;14:180.
67. Erlich JM, Talmor DS, Cartin-Ceba R, et al. Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury: a population-based cohort study. Chest. 2011;139:289–95.
68. Hess R, Wujak L, Hesse C, et al. Coagulation factor XII regulates inflammatory responses in human lungs. Thromb Haemost. 2017;10:1896–907.
69. Igonin AA, Protsenko DN, Galstyan GM, et al. C1-esterase inhibitor infusion increases survival rates for patients with sepsis. Crit Care Med. 2012;40:770–7.
70. Tomoharu M, Ali KA, Shinichi N, et al. A three-phase approach for the early identification of acute lung injury induced by severe sepsis. In Vivo. 2016;30:341–50.
71. Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. Arch Pathol Lab Med. 2016;140(4):345–50.
72. Wheeler AP, Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. Lancet. 2007;369:1553–65.
73. Hooper RG, Kearl RA. Established ARDS treated with a sustained course of adrenocorticosteroids. Chest. 1990;97:138–43.
74. Steinberg KP, Hudson LD, Goodman RB, et al. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med. 2006;354:1671–84.
75. Iwata K, Doi A, Ohji G, et al. Effect of neutrophil elastase inhibitor (sivelestat sodium) in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): a systematic review and meta-analysis. Intern Med. 2010;49:2423–32.
76. Orihara K, Matsuda A. Pathophysiological roles of microvascular alterations in pulmonary inflammatory diseases: possible implications of tumor necrosis factor-alpha and CXC chemokines. Int J Chron Obstruct Pulmon Dis. 2008;3(4):619–27.
77. Takano Y, Mitsuhashi H, Ueno K. Alpha, 25-Dihydroxy vitamin D(3) inhibits neutrophil recruitment in hamster model of acute lung injury. Steroids. 2011;76:1305–9.
78. Parekh D, Dancer RC, Lax S, et al. Vitamin D to prevent acute lung injury following oesophagectomy (VINDALOO): study protocol for a randomised placebo controlled trial. Trials. 2013;14:100.
79. Sadowitzaa B, Royaa S, Gattobb LA. Lung injury induced by sepsis: lessons learned from large animal models and future directions for treatment. Expert Rev Anti Infect Ther. 2011;9(12):1169–78.
80. Bein T, Grasso S, Moerer O, et al. The standard of care of patients with ARDS: ventilatory settings and rescue therapies for refractory hypoxemia. Intensive Care Med. 2016;42(5):699–711.
81. Esteban A, Anzueto A, Frutos F, et al. Characteristics and outcomes in adult patients receiving mechanical ventilation: a 28 day international study. JAMA. 2002;287:345–55.
82. Frank JA, Gutierrez JA, Jones KD, et al. Low tidal volume reduces epithelial and endothelial injury in acid-injured rat lungs. Am J Respir Crit Care Med. 2002;165:242–9.
83. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med. 2005;33:1–6.
84. Pepe PE, Hudson LD, Carrico JC. Early application of positive end-expiratory pressure in patients at risk of adult respiratory distress syndrome. N Engl J Med. 1984;311:281–6.
85. Crotti S, Mascheroni D, Caironi P, et al. Recruitment and derecruitment during acute respiratory failure: a clinical study. Am J Respir Crit Care Med. 2001;164:131–40.
86. Daoud EG, Farag HL, Chatburn RL, et al. Airway pressure release ventilation: what do we know? Respir Care. 2012;57(2):282–92.
87. Marcelo BP, Amato MD, Maureen O, et al. Driving pressure and survival in the acute respiratory distress syndrome. N Engl J Med. 2015;372(8):747–55.
88. Guerin C, Reignier J, Richard JC, et al. Prone positioning in severe acute respiratory distress syndrome. N Engl J Med. 2013;368(23):2159–68.

89. Cressoni M, Chiumello D, Algieri I, et al. Opening pressures and atelectrauma in acute respiratory distress syndrome. Intensive Care Med. 2017;43(5):603–11.
90. Sklar MC, Fan E, Goligher EC. High-frequency oscillatory ventilation in adults with ards: past, present, and future. Chest. 2017;3(17):31185–6.
91. Umbrello M, Marino A, Chiumello D. Tidal volume in acute respiratory distress syndrome: how best to select it. Ann Transl Med. 2017;5(14):287.
92. Del Sorbo L, Cypel M, Fan E, et al. Extracorporeal life support for adults with severe acute respiratory failure. Lancet Respir Med. 2014;2(2):154–64.
93. Abrams D, Brodie D. Extracorporeal membrane oxygenation for adult respiratory failure: 2017 update. Chest. 2017;152(3):639–49.
94. Vaquer S, de Haro C, Peruga P, et al. Systematic review and meta-analysis of complications and mortality of veno-venous extracorporeal membrane oxygenation for refractory acute respiratory distress syndrome. Ann Intensive Care. 2017;7(1):51–64.
95. Leligdowicz A, Fan E. Extracorporeal life support for severe acute respiratory distress syndrome. Curr Opin Crit Care. 2015;21(1):13–9.
96. Combes A, Pesenti A, Ranieri VM. Fifty years of research in ARDS. Is extracorporeal circulation the future of acute respiratory distress syndrome management. Am J Respir Crit Care Med. 2017;195(9):1161–70.
97. Ragaller M, Bleyl JU, Koch T, et al. From isoflurane to perfluorohexane? PerfluorocarbonsDOUBLEHYPHENtherapeutic strategies in acute lung failure. Anaesthesist. 2000;49(4):291–301.
98. Levy SD, Alladina JW, Hibbert KA, et al. High-flow oxygen therapy and other inhaled therapies in intensive care units. Lancet. 2016;387(10030):1867–78.
99. Beitler JR, Goligher EC, Schmidt M, et al. Personalized medicine for ARDS: the 2035 research agenda. Intensive Care Med. 2016;42(5):756–67.
100. Hashimoto S, Sanui M, Egi M, et al. The clinical practice guideline for the management of ARDS in Japan. J Intensive Care. 2017;5:50–82.
101. Wang L, Bastarache JA, Wickersham N, et al. Novel role of the human alveolar epithelium in regulating intra-alveolar coagulation. Am J Respir Cell Mol Biol. 2007;36:497–503.
102. Robriquet L, Collet F, Tournoys A, et al. Intravenous administration of activated protein C in pseudomonas induced lung injury: impact on lung fluid balance and the inflammatory response. Respir Res. 2006;7:41.
103. Healy LD, Puy C, Fernández JA, et al. Activated protein C inhibits neutrophil extracellular trap formation in vitro and activation in vivo. J Biol Chem. 2017;292(21):8616–29.
104. Cornet AD, Groeneveld AB, Hofstra JJ, et al. Recombinant human activated protein C in the treatment of acute respiratory distress syndrome: a randomized clinical trial. PLoS One. 2014;9(3):90983–94.
105. Christiaans SC, Wagener BM, Esmon CT, et al. Protein C and acute inflammation: a clinical and biological perspective. Am J Physiol Lung Cell Mol Physiol. 2013;305(7):L455–66.
106. Ma J, Bai J. Protective effects of heparin on endothelial cells in sepsis. Int J Clin Exp Med. 2015;8(4):5547–52.
107. Horie S, Masterson C, Devaney J, et al. Stem cell therapy for acute respiratory distress syndrome: a promising future. Curr Opin Crit Care. 2016;22(1):14–20.
108. Islam MN, Das SR, Emin MT, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med. 2012;18:759–65.
109. Johnson CL, Soeder Y, Dahlke MH. Concise review: mesenchymal stromal cell-based approaches for the treatment of acute respiratory distress and sepsis syndromes. Stem Cells Transl Med. 2017;6(4):1141–51.
110. Matthay MA, Goolaerts A, Howard JP, et al. Mesenchymal stem cells for acute lung injury: preclinical evidence. Crit Care Med. 2010;38(10):569–73.
111. Lee JW, Krasnodembskaya A, McKenna DH, et al. Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. Am J Respir Crit Care Med. 2013;187:751–60.

112. Keane C, Jerkic M, Laffey JG. Stem cell-based therapies for sepsis. Anesthesiology. 2017;127(6):1017–34.
113. Horák J, Nalos L, Martínková V, et al. Mesenchymal stem cells in sepsis and associated organ dysfunction: a promising future or blind alley? Stem Cells Int. 2017;2017:1–10.
114. Zheng G, Huang L, Tong H, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. Respir Res. 2014;15:39.
115. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respir Med. 2015;3:24–32.
116. Liu KD, Wilson JG, Zhuo H, et al. Design and implementation of the START (STem cells for ARDS treatment) trial, a phase 1/2 trial of human mesenchymal stem/stromal cells for the treatment of moderate-severe acute respiratory distress syndrome. Ann Intensive Care. 2014;4:22.
117. Zhou J, Wu Y, Henderson F, et al. Adenoviral gene transfer of a mutant surfactant enzyme ameliorates pseudomonas-induced lung injury. Gene Ther. 2006;13:974–85.
118. Reiss LK, Schuppert A, Uhlig S. Inflammatory processes during acute respiratory distress syndrome: a complex system. Curr Opin Crit Care. 2017;23:1–9.
119. Huang Y, Sauthoff H, Herscovici P, et al. Angiopoietin-1 increases survival and reduces the development of lung edema induced by endotoxin administration in a murine model of acute lung injury. Crit Care Med. 2008;36:262–7.
120. Bromberg Z, Raj N, Goloubinoff P, et al. Enhanced expression of 70-kilodalton heat shock protein limits cell division in a sepsis-induced model of acute respiratory distress syndrome. Crit Care Med. 2008;36:246–55.
121. Li Y, Dong J, Wu M. Human Apo A-I overexpression diminishes LPS-induced systemic inflammation and multiple organ damage in mice. Eur J Pharmacol. 2008;590:417–22.
122. Shu Q, Shi Z, Zhao Z, et al. Protection against Pseudomonas aeruginosa pneumonia and sepsis-induced lung injury by overexpression of β-defensin-2 in rats. Shock. 2006;26:365–71.
123. Han J, Lu X, Zou L, et al. E-prostanoid 2 receptor overexpression promoted mesenchymal stem cell attenuated lung injury. Hum Gene Ther. 2016;27:1–10.
124. Mei SH, Dos Santos CC, Stewart DJ. Advances in stem cell and cell-based gene therapy approaches for experimental acute lung injury: a review of preclinical studies. Hum Gene Ther. 2016;27(10):802–12.
125. Chen G, Xu Y, Jing J, et al. The anti-sepsis activity of the components of Huanglian Jiedu Decoction with high lipid A-binding affinity. Int Immunopharmacol. 2017;46:87–96.
126. Ding XM, Pan L, Wang Y, et al. Baicalin exerts protective effects against lipopolysaccharide-induced acute lung injury by regulating the crosstalk between the CX3CL1- CX3CR1 axis and NF-κB pathway in CX3CL1-knockout mice. Int J Mol Med. 2016;37(3):703–15.
127. Huang K-L, Chen C-S, Hsu C-W, Li M-H, Chang H, Tsai S-H, Chu S-J. Therapeutic effects of baicalin on lipopolysaccharide-induced acute lung injury in rats. Am J Chin Med. 2008;36(02):301–11.
128. Gu XH, Xu LJ, Liu ZQ, et al. The flavonoid baicalein rescues synaptic plasticity and memory deficits in a mouse model of Alzheimer's disease. Behav Brain Res. 2016;311:309–21.
129. Tsai CL, Lin YC, Wang HM, et al. Baicalein, an active component of scutellaria baicalensis, protects against lipopolysaccharide-induced acute lung injury in rats. J Ethnopharmacol. 2014;153(1):197–206.
130. Wang L, Chen J, Wang B, et al. Protective effect of quercetin on lipopolysaccharide-induced acute lung injury in mice by inhibiting inflammatory cell influx. Exp Biol Med. 2014;239(12):1653–62.
131. Takashima K, Matsushima M, Hashimoto K, et al. Protective effects of intratracheally administered quercetin on lipopolysaccharide-induced acute lung injury. Respir Res. 2014;15(1):150–60.
132. Huang R, Zhong T, Wu H. Experimental research Quercetin protects against lipopolysaccharide-induced acute lung injury in rats through suppression of inflammation and oxidative stress. Arch Med Sci. 2015;11(2):427–32.

133. Kumari A, Tyagi N, Dash D, et al. Intranasal curcumin ameliorates lipopolysaccharide-induced acute lung injury in mice. Inflammation. 2015;38(3):1103–12.
134. Xin W, Zhang L, Fan H, et al. Escin attenuates acute lung injury induced by endotoxin in mice. Eur J Pharm Sci. 2011;42(1–2):73–80.
135. Qiu J, Yu L, Zhang X, et al. Asiaticoside attenuates lipopolysaccharide-induced acute lung injury via down-regulation of NF-κB signaling pathway. Int Immunopharmacol. 2015;26(1):181–7.
136. Guan S, Xiong Y, Song B, et al. Protective effects of salidroside from Rhodiola rosea on LPS-induced acute lung injury in mice. Immunopharmacol Immunotoxicol. 2012;34(4):667–72.
137. Lu R, Wu Y, Guo G, et al. Salidroside protects lipopolysaccharide-induced acute lung injury in mice. Dose Response. 2016;14(4):1–5.
138. Li MH, Kothandan G, Cho SJ, et al. Magnolol inhibits LPS-induced NF-κ B/Rel activation by blocking p38 kinase in murine macrophages. Korean J Physiol Pharmacol. 2010;14(6):353–8.
139. Fu Y, Liu B, Feng X, et al. The effect of magnolol on the toll-like receptor 4/nuclear factor kappa B signaling pathway in lipopolysaccharide-induced acute lung injury in mice. Eur J Pharmacol. 2012;689(1):255–61.
140. Zhou H, Bian D, Jiao X, et al. Paeoniflorin protects against lipopolysaccharide-induced acute lung injury in mice by alleviating inflammatory cell infiltration and microvascular permeability. Inflamm Res. 2011;60(10):981–90.
141. Cao Q, Jing C, Tang X, et al. Protective effect of resveratrol on acute lung injury induced by lipopolysaccharide in mice. Anat Rec. 2011;294(3):527–32.
142. Li T, Zhang J, Feng J, et al. Resveratrol reduces acute lung injury in a LPS induced sepsis mouse model via activation of Sirt1. Mol Med Rep. 2013;7(6):1889–95.
143. Zhang Z, Chen N, Liu JB, et al. Protective effect of resveratrol against acute lung injury induced by lipopolysaccharide via inhibiting the myd88 dependent Toll-like receptor 4 signaling pathway. Mol Med Rep. 2014;10(1):101–6.
144. Bae HB, Li M, Kim JP, et al. The effect of epigallocatechin gallate on lipopolysaccharide-induced acute lung injury in a murine model. Inflammation. 2010;33(2):82–91.
145. Liu W, Dong M, Bo L, Li C, Liu Q, Li Y, Ma L, Xie Y, Fu E, Mu D, Pan L, Jin F, Li Z. Epigallocatechin-3-gallate ameliorates seawater aspiration-induced acute lung injury via regulating inflammatory cytokines and inhibiting JAK/STAT1 pathway in rats. Mediators Inflamm. 2014;2014:1–12.

Progress of Research in Neonatal Sepsis

16

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Abstract

Neonatal sepsis remains a significant global problem with little progress made despite major efforts. At present, there is a lack of an accepted international consensus on the definition, diagnosis, and treatment of neonatal sepsis; the unclear understanding of the pathogenesis of neonatal sepsis leads to blindness in treatment, which will result in an unsatisfactory therapeutic outcome. In addition, some serious diseases caused by noninfectious factors, such as trauma, stress, asphyxia, and so on, have very similar pathophysiological results with neonatal sepsis. In this review we synthesize the recent advances in definition, incidence, causative agents, risk factors, pathophysiology, clinical manifestations, and diagnosis and treatment of neonatal sepsis. Of course, there are still many challenges to neonatal sepsis in many ways.

Keywords

Pediatric · Neonate · Sepsis · Septic shock · Definition · Surviving sepsis campaign Antibiotics · Burden · Causative agent · Risk factor · Diagnosis · Management

16.1 Introduction

Neonatal sepsis remains the third leading cause of neonatal death and is one of the leading causes of death among children under 5 years of age, especially in developing countries, which has become a public health problem. The clinical

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manifestations of neonatal sepsis range from subclinical infection to severe focal or systemic diseases. Pathogens can come from intrauterine infections, infections of maternal flora, or hospital- or community-acquired infections. Preterm infants are immunologically immature. During the period of hospitalization, more invasive procedures during hospitalization increase the chances of bacterial infection. However, in many cases, serious diseases caused nonpathogenic bacteria, such as trauma, stress, asphyxia, hypoxia, and so on, have similar pathophysiological processes with neonatal sepsis, which is also worthy of our study. In this review, we discussed the most common problems and challenges in the definition, burden, etiology, risk factors, pathophysiology, diagnosis, and treatment of neonatal sepsis, with a focus on developing countries.

16.2 Definition of Neonatal Sepsis

Since the American College of chest Physicians/Society of critical Care Medicine (ACCP/SCCM) proposed the concept of sepsis in 1991, experimental and clinical studies on sepsis have been in the ascendant. The first edition of sepsis in 1991 was defined as systemic inflammatory response syndrome (SIRS) caused by infection. The SIRS is identified by two or more symptoms including fever or hypothermia, tachycardia, tachypnea, and change in blood leucocyte count; the sepsis-1 emphasizes that the direct factor leading to body damage is immune response rather than pathogens. In December 2001, an international conference on sepsis was held in Washington, USA. The definition and diagnostic criteria of sepsis proposed by ACCP/SCCM in 1991 were discussed and re-evaluated. The meeting concluded that the previous criteria for the diagnosis of sepsis were too loose, with a high sensitivity and low specificity, which may lead to overdiagnosis of sepsis. As a result, the second definition of sepsis (sepsis-2) has been revised, and more stringent diagnostic criteria have been introduced than in the past. However, the clinical application of modified sepsis is limited due to the complexity of the diagnostic criteria. Both the "Sepsis syndrome" proposed by Bone and the definition of sepsis proposed at the ACCP/SCCM meeting in 1991 are all aimed at adults, such as the heart rate and respiratory rate in SIRS are the standard of adults, so these concepts have not been applied to children. In 2016, a sepsis task force was established and re-evaluated the definition of sepsis. According to the newest definitions (sepsis-3), sepsis should be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock should be defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone [1]. Unfortunately, to this day, there is no precise definition of neonatal sepsis. But according to the latest definition, neonatal sepsis also contains three characteristics, the first of which emphasizes infection; neonatal sepsis can be caused by bacteria, viruses, or fungi (yeast); however, many neonatal sepsis is not entirely caused by infection; some noninfectious factors such as asphyxia, trauma, and stress can also be associated with clinical sepsis. The second characteristic is the dysregulated host response, which is a key factor leading to hemodynamic changes and other organ damage. The third characteristic is a

life-threatening organ dysfunction, which emphasizes the consequences of sepsis. Among these three characteristics, we should pay more attention to host's own immune disorder, because it is the central link of neonatal sepsis, but also the key to prevention and control. According to the age of onset and timing of the sepsis episode, it can be divided into two types: early-onset sepsis (EOS) and late-onset sepsis (LOS). The clinical manifestations of EOS usually occur in the first 72 h of life, most of the symptoms occur within 24 h of birth. Some clinicians define EOS, especially those caused by group B Streptococcus (GBS), as infections that occur less than 7 days of age. EOS is acquired before or during childbirth and is usually transmitted vertically from mother to child. Pathogens enter the fetus through the respiratory tract (fetal respiration), gastrointestinal tract (swallowing), skin, ears, etc. LOS refers to sepsis presenting after 72 h of life, and pathogens invade newborns through medical personnel, family members, contaminated equipment, etc.

16.3 Burden of Neonatal Sepsis

In 2010 worldwide, 7.6 million children less than 5 years old died, predominantly due to infectious causes including sepsis; neonatal deaths accounted for 40% of the total lives lost [1]. In 1990, both the United Nations (UN) and World Health Organization (WHO) prioritized a second/third reduction in the unacceptable child mortality rate by 2015. However, in 2013, 44% of deaths in children under the age of 5 occurred during the neonatal period, up from 37% in 1990. Despite major advances in neonatal care and increasing research, in developed countries, four of every ten infants with sepsis die or experience major disability including significant permanent neurodevelopmental impairment [2]. Prematurely born neonates experience the highest incidence and mortality of sepsis among all age groups [3, 4]. In the United States, a staggering 36% of neonates born before 28 weeks completed gestation suffer at least one episode of bloodstream infection (BSI) during their birth hospitalization with up to a 50% associated mortality [5]. Compared to term infants, sepsis in preterm infants is up to 1000-fold more common and is associated with higher rates of mortality and lifelong neurodevelopmental handicaps [6, 7]. Of note, it is estimated that 11% of the 135 million births globally occur before 37 weeks of gestation, and preterm births have been increasing steadily, especially in developed countries [8]. In 2005, there were 41,353 newborns from 80 hospitals in China, with 2060 cases of sepsis. The incidence of sepsis was about 5%.

16.4 Pathogens of Neonatal Sepsis

16.4.1 EOS

Intrauterine infection is a serious complication of pregnancy, which can lead to stillbirth and premature delivery and significantly increase the morbidity and mortality of neonatal sepsis and brain injury. Under normal circumstances, the amniotic cavity is usually sterile. There are a large number of aerobic and anaerobic bacteria

in the human birth canal, which can lead to infection of newborn before or during delivery under different pathological conditions, the most common pathway is the ascending invasion of microbes from the lower reproductive tract. Romero et al. ascending intrauterine infection is divided into four phases. Stage I: Disorder of vaginal and/or cervical microbiota or presence of pathogenic microorganisms in the cervix canal. Bacterial vaginosis may be an early manifestation of this period. Stage II: Microorganisms enter the uterine cavity and colonize in the decidual tissue and then cause inflammation, which in turn develops into chorioamnionitis. Stage III: Microorganisms continue to invade and colonize in the chorion and amniotic membrane. Later, the pathogens may invade the fetal blood circulation and lead to chorioangiitis and/or umbilical phlebitis; pathogens can also cross amniotic membranes into the amniotic cavity. Stage IV: The fetus is infected by direct contact or inhalation, leading to otitis, conjunctivitis, umbilical inflammation, congenital pneumonia, etc. Chorioamnionitis is an inflammatory reaction of the chorion, and neutrophils infiltration is the main pathological feature. Chorioamnionitis is divided into two types: acute chorioamnionitis (ACAM) with clinical symptoms including fever, maternal or fetal tachycardia, uterine tenderness, and amniotic fluid odor and histologic chorioamnionitis (HCAM) without clinical symptoms. The latter was only found in microscopic examination of placental pathology. The incidence of the latter was two to three times higher than that of ACAM. In addition, newborns may be infected when exposed to potentially pathogenic bacteria, viruses, or fungi through the birth canal. The incidence of histological chorioamnionitis is negatively correlated with gestational age and was directly related to duration of membrane rupture [3, 4]. Whether asymptomatic neonates with gestational age $\geq$ 35 weeks should be treated with empirical antibiotics due to maternal chorioamnionitis is still controversial. A recent study showed that the use of antibiotics in asymptomatic newborns exposed to chorioamnionitis should be evaluated comprehensively on the basis of maternal risk factors and neonatal clinical examinations; with maternal body temperature as a continuous variable for risk factors, the clinical examination of newborn mainly includes blood culture, blood cell count, and hsCRP. Simple observation without laboratory tests may miss some asymptomatic children.

16.4.2 LOS

The neonatal immune system is not mature enough and the function is not perfect; the innate immune system, including phagocytes, antigen-presenting cells, and natural killer cells, and the complement system provide a defense against pathogens. Decreased function of neutrophils and low concentrations of immunoglobulins increase the susceptibility of preterm infants to invasive infection. As infants age, they are exposed to environmental organisms that might be pathogenic. Contact with family members, hospital personnel, contaminated equipment, and nutritional sources all increase the probability of disease exposure. Twenty to 40% of nosocomial infections are related to doctors' hand contamination. Despite this importance, hand hygiene has not received sufficient attention. Other risk factors for LOS

include intravascular catheterization, delayed enteral nutrition, prolonged parenteral nutrition, prolonged mechanical ventilation, surgery, etc. Most cases of meningitis are late-onset infections resulting from hematogenous spread via the choroid plexus into the CNS; less often, late-onset meningitis results from contiguous spread as a result of contamination of open neural tube defects, ventricular devices, congenital sinus tracts, or penetrating wounds from fetal scalp monitors. Subdural effusion, ventriculitis, hydrocephalus, abscess formation, and septic infarcts are main complications of neonatal meningitis.

16.4.3 Causes of Neonatal Sepsis

The causative agent of neonatal sepsis may be bacteria, viruses, or fungi microorganisms. The most common pathogens causing EOS are *Streptococcus agalactiae* (GBS) and *Escherichia coli.* Of the nearly 400,000 live births from 2006 to 2009 at the academic Neonatal Center in the United States, 389 newborns had early-onset infections (0.98 infections per 1000 live births), of which 43% were GBS (0.41 per 1000 live births) and 29% were *E. coli* (0.28 per 1000 livebirths). Most of the newborns infected with GBS were term infants, while those infected with *E. coli* were more common in premature infants. The infection rate was negatively correlated with birth weight: 54% at 22–24 weeks, 30% at 25–28 weeks, 12% at 29–33 weeks, and 3% at more than 37 weeks' gestation. The mortality rates of newborns infected with GBS and *Escherichia coli* were 9% and 33%, respectively. In Europe or North America, GBS used to be the most common pathogen of premature infections, but at present *E. coli* almost replaced GBS as the most important pathogen associated with early-onset infection in preterm infants and very low birth weight infants [3, 9, 10]. However, in China, GBS infection is not as serious as that in Europe and America; the most common pathogens include coagulase-negative staphylococci (69.2%) and *Escherichia coli* (15.5%). Except for GBS and *Escherichia coli*, some unusual pathogens such as *Listeria monocytogenes*, non-typeable *Haemophilus influenzae*, Gram-negative enteric bacilli, and *Candida* spp. can also be pathogenic bacteria of EOS [11, 12]. In China LOS can also be associated with *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungal. However, *L. monocytogenes* is very rare. In neonatal intensive care units, *Coagulase negative staphylococci* are also the most common pathogens of LOS. *Coagulase-negative staphylococci* and *Staphylococcus aureus* are most commonly in neonates with vascular access catheters. For example, of the 117 infants with *Staphylococcus aureus* septicemia in 13 neonatal units in the United Kingdom, 8 (7%) were caused by *methicillin-resistant S. aureus* (MRSA). The mean gestational age and birth weight were 27 weeks and 850 g, respectively. The incidence of *Staphylococcus aureus* sepsis in live births and infants less than 1500 g was 0.6 per 1000 and 23 per 1000, respectively. Ninety-four of the cases were late-onset infection, which occurred more than 48 h; all of the seven episodes categorized as early-onset were MRSA infection. Half of the infants showed non-localizing signs of sepsis, and half of the infants had central venous access when they were infected with *Staphylococcus aureus* [13]. Other rare

pathogens of early and late sepsis are *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Neisseria gonorrhoeae*. Additionally, *Neisseria meningitidis*, *Mycoplasma hominis*, and *Ureaplasma* spp. have been associated with early-onset sepsis, pneumonia, meningitis, cerebral abscesses, and osteomyelitis. The prevalence of pathogens varies greatly from region to region, and Gram-negative bacteria are a significant burden in resource-poor areas [14]. Viruses can also cause neonatal sepsis, most commonly herpes simplex virus (HSV) and enterovirus, both of which are more associated with late-onset sepsis. Neonatal HSV infection has a high incidence and mortality, which may be located in the eyes and mouth, skin, involve the central nervous system, or spread to the liver, lungs, and adrenal glands. The onset time was 5–9 days. Neonatal HSV infection can be caused by HSV-1 or HSV-2; HSV-1 will become more common with the increase of HSV-1 genital infection [15, 16]. Neonates with enterovirus infections might develop myocarditis, meningoencephalitis, and hepatitis, following fever, lethargy, poor feeding, irritability, jaundice, and hypoperfusion. Infants younger than 10 days of age who are exposed to echoviruses, coxsackie group B viruses, and parechoviruses through maternal shedding are not normally able to benefit from transplacental transfer of maternal antibodies because of their inability to produce an immune response and because of the timing of recent maternal infections [17]. Fungi, particularly yeasts, are associated with a growing number of systemic infections, usually acquired during prolonged hospitalization of premature infants. *Candida* spp. are the third most common cause of late-onset neonatal sepsis in low birth weight infants (<1500 g), and *Candida parapsilosis* is a main pathogen in neonates with central venous access. The incidence of *C. parapsilosis* infection is relatively low in Europe compared with North America and Australia [18]. Preterm infants, especially very low birth weight infants, have low immune function, which is the main risk group of neonatal fungal infection. Other risk factors included prolonged intensive care, parenteral nutrition, mechanical ventilation, central venous catheterization, prolonged use of broad-spectrum antibiotics and H2 receptor blockers, and postpartum corticosteroids. In a prospective observational cohort of 1515 infants with 1000 g birthweight or less who were from 19 academic medical centers in the United States, invasive candidiasis occurred in 137 (9%). Potentially modifiable risk factors included receipt of broad-spectrum and antenatal antibiotics including third-generation cephalosporins, central venous catheters, receipt of intravenous lipid emulsion, antacid medications, postnatal corticosteroids, and the presence of an endotracheal tube [19].

16.5 Risk Factors

16.5.1 EOS

16.5.1.1 Maternal Factors

Premature birth (<37 weeks), prolonged time (>18 h) of membranes rupture, maternal peripartum infection, and low socioeconomic status are closely related to EOS. A study [20] differentiated the categories of predisposing factors into the following: maternal colonization, risk factors for infection, and maternal infection.

Maternal colonization was determined that the reproductive tract/genital bacterial cultures were positive no matter whether the pregnant women had clinical signs or symptoms or not. Maternal risk factors included prelabor rupture of membranes during the term of the fetus, preterm prelabor rupture of membranes, and duration of rupture of membranes longer than 8–24 h or undefined. They defined maternal infection according to the following criteria: clinical signs of infection [uterine tenderness, intrapartum maternal fever, maternal tachycardia, malodorous vaginal discharge, elevated C-reactive protein (CRP), elevated white cell count, physician diagnosis of clinical chorioamnionitis] or the presence of laboratory confirmed bacterial infection [bacteremia, urinary tract infections, amnionitis, or chorioamnionitis; documented by positive polymerase chain reaction (PCR) at the level of the amniotic fluid only; positive cultures of biologic fluids; or histopathologically confirmed chorioamnionitis], and the multivariate logistic regression analysis of a Chinese 1:4 case-control study [21] involving 147 EOS newborns and 588 controls showed that cesarean section (OR 0.103, 95%CI, 0.041–0.258), maternal age >35 years [odd ratio (OR) 4.835, 95% confidence interval (CI), 1.170–19.981], and premature rupture of membranes (OR 0.207, 95%CI, 0.078–0.547) represent the major predisposing factors to neonatal sepsis. Furthermore, in the univariate analysis, urban residence (OR 5.079, 95%CI, 2.899–8.990), fixed occupation of mothers (OR 0.439, 95%CI, 0.289–0.668), abnormal fetal position (OR 1.621, 95%CI, 1.340–1.962), parity (OR 1.859, 95%CI, 1.188–2.908), fetal times (OR 1.212, 95%CI, 1.041–1.412), amniotic fluid volume abnormalities (OR 0.200, 95%CI, 0.054–0.745), placental abnormalities (OR 0.050, 95% CI, 0.006–0.428) pregnancy-induced hypertension (OR 0.297, 95%CI, 0.122–0.726), and pregnancy-induced hypertension (OR 0.297, 95%CI, 0.122–0.726) seemed to predispose to neonatal infection; unfortunately these results were not confirmed by multivariate regression analysis evaluation. The role of young maternal age (<20 years old) has been questioned, although it was previously thought to be an important predisposing factor of neonatal sepsis, possibly related to the high colonization rate of GBS in the maternal vagina [22]. Epidemiological studies have shown an increase in the incidence of EOS among black newborns compared with white newborns, although this explanation seems to be better related to the different socioeconomic conditions of the two races [23]. Certain obstetric practices such as membrane-stripping, invasive fetal monitoring, and intrapartum vaginal exams may be important causes of early neonatal infection [24].

16.5.1.2 Neonatal Factors

Among neonatal factors able to promote EOS, the alterations of the innate immune response can play a significant role. As the adaptive response requires 5–7 days from delivery to develop, during this period infants are largely dependent on innate immune system (respiratory and intestinal) barriers and the skin, local immune sentinel cells, [macrophages, endothelium, epithelium, polymorphonuclear cells (PMN), and dendritic cells], antigen presenting immune cells (monocytes, macrophages, and dendritic cells), host defense proteins and peptides (complements, cytokines, chemokines, active phase, and coagulation proteins), as well as

passively acquired immunoglobulin from the mother. The alterations of the innate immune can play an important role in neonatal EOS. Because the infant's adaptive response will take 5–7 days to form, during that time, infants mainly rely on innate immune system (intestinal and respiratory) barrier and the skin, local immune sentinel cells [polymorphonuclear cells (PMN), macrophages epithelial cells, dendritic cells, and endothelial cells], antigen immune cells (macrophages, monocytes, and dendritic cells), host defense proteins and peptides (cytokines, complements, active and clotting proteins, chemokines), and immunoglobulins acquired passively by the mother. Deficiencies in immunomodulatory genes (mainly X-linked genes) and premature infants (especially low birth weight) are to be closely related to innate immaturity and/or function of the immune system, thus increasing the likelihood of infections [25]. Birth weight is also a risk factor for EOS; premature infants, especially VLBW, are ten times more likely than those born at term, with an overall mortality rate of about one-third [26]. In addition, prematurity (OR 0.059, 95%CI, 0.010–0.329) and newborn jaundice (OR 0.092, 95%CI, 0.021–0.404) were also sensitive to EOS in a multivariate analysis of a recent case-control study [21]. Other neonatal risk factors include neonatal Apgar scoring at 1 and at 5 min, male sex, wet lung, anemia, fetal distress, hypothermia, intraventricular hemorrhage, and metabolic disorders [20].

16.5.2 LOS

A review of studies from the NICHD Neonatal Research Network showed that the likelihood of developing LOS was inversely proportional to birth weight and gestational age [401–750 g (43%) and highest in infants <25 weeks gestation (46%)] [27]. Moreover, while maternal intake of corticosteroids was closely related to a significant reduction in EOS (unadjusted OR 0.52; 95%CI, 0.31–0.88), it was also related to an increased risk of LOS (unadjusted OR 1.29; 95% CI, 1.10–1.51). However, the increased incidence of LOS in newborns having undergone antenatal administration of corticosteroids must be balanced with the significant reduction in respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, death rates, and risk of EOS observed after corticosteroids use [27]. A Swedish retrospective case-control study demonstrated that the risk of LOS was directly related to ventilatory treatment and duration of central/umbilical catheters (OR 1.6 and OR 2.6, respectively). Premature rupture of membranes, fever during delivery, and days of continuous positive airway pressure treatment seem to not be relevant to LOS (p = not significant) [28]. A study of 164 Taiwanese infants with bloodstream infection showed that intraventricular hemorrhage (OR 2.68, 95% CI, 1.20–5.99; P = 0.017) and parenteral nutrition (OR 6.07, 95% CI, 1.14–32.32; P = 0.034) were independently associated with blood flow infection. In addition, a retrospective cohort study of NICUS patients using peripherally inserted central catheters from 2003 to 2010 showed that catheter removal due to adverse events was significantly associated with LOS and the use of antibiotics before extubation was not associated with a decrease in the incidence of sepsis [29].

16.6 Pathophysiology of Neonatal Sepsis

16.6.1 Host's Own Immune Damage

Sepsis is mainly caused by host's own uncontrolled inflammation. This means that infections cause damage not only because of the virulence of the pathogen but also because of the host's reaction. When bacteria, fungi, viruses, and bacterial toxins penetrate into the body, they will eventually be discovered by the innate immune system. Innate immunity is the ensemble of cellular and humoral mechanisms that does not need training (i.e., previous exposure to a germ) and that will act quasi-automatically against an insult and generate inflammation in a nonspecific manner [30, 31]. And when the immune inflammatory response is out of control, the release of inflammatory cytokines will induce the production and release of new cytokines; it causes "cytokine storm" and "cytokine waterfall," which can result in cell and organ damage. The cytokines are a broad category of relatively small proteins (<40 kDa). They cover autocrine, paracrine, and endocrine activities and play an important immunomodulating function. The cytokines can be divided into several categories: chemokines, interleukins, interferons, tumor necrosis factor, and growth factors. The cytokines induced by infection is the result of an autoamplifying phenomenon aiming to destroy the invader and restore the balance of immune system, meanwhile the cytokines can activate neuroendocrine reflex and plasma protein cascade systems such as coagulation, fibrinolysis, and complement systems. Eventually, when a threshold is crossed, a severe clinical syndrome, "sepsis," can occur.

16.6.2 Microcirculation Dysfunction

Microcirculation consists of vessels less than 100 μm in diameter where oxygen release to the tissues takes place and consists of arterioles, capillaries, and venules. The main cell types comprising the microcirculation are the endothelial cells, smooth muscle cells (mostly in arterioles), leukocytes, red blood cells, and plasma components in blood. Normal function of microcirculation is the main prerequisite for adequate tissue oxygenation and thus organ function. Its function is to transport oxygen and nutrients to tissue cells, ensure adequate immune function, and, in disease, deliver therapeutic drugs to target cells. Although microcirculatory changes in sepsis have been recognized from long ago, clinical interest in this field has grown over the last decade with the development of bedside video microscopic techniques, which confirms that similar microcirculatory disorders can be observed in septic patients. The existence and persistence of this abnormality are related to the prognosis. A study [32] reported that compared with healthy volunteers, sepsis patients had significantly lower vascular density and the proportion of small perfusion vessels, with microcirculatory disturbance more severe in non-survivors; similar results were later confirmed by TreCeik et al. [33]. In addition, Trzeciak also found that the changes of microcirculation were closely related to the severity of organ failure. Sakr et al. [34] has characterized the relationship between the time course of

microcirculation changes and prognosis in patients with septic shock. Although similar at baseline, the microcirculation of survivors improves rapidly compared with non-survivors, although there was no difference in global hemodynamic variables. More than that, capillary perfusion when shock resolved was closely related to the severity of organ failure. However, the reported statistical association between microcirculatory abnormalities and outcome does not predicate a mechanistic relation. Microcirculatory alterations during sepsis may involve many mechanisms and are related to all the components of the microcirculation [35, 36]: redistribution of blood flow from compliant vascular beds (the splanchnic area and skin) to more important body areas (heart, brain), with secondary microvascular derecruitment; endothelial activation and injury; loss of the glycocalyx, which covers the endothelium and forms an important barrier and transduction system; increased microvascular permeability with capillary leakage, edema formation, and hypovolemia; production of reactive oxygen species (ROS); increased leukocyte adhesion to the endothelial surface; decreased RBC deformability, with secondary capillary plugging that directly damages microcirculatory structures, cellular interactions, and hemostasis; damaged arteriolar smooth muscle cell tone and reactivity, secondary to dysregulation of NO production; and capillary obstruction by platelet/fibrin clots secondary to disseminated intravascular coagulation. The role of endothelial dysfunction, and the consequent increase in leukocyte and platelet adhesion, has recently been considered as a potential mechanism contributing to microcirculatory flow abnormalities [37]. Increased expression of adhesion molecules on endothelial cells and immune cells has been demonstrated during sepsis.

16.6.3 Mitochondrial Dysfunction

Mitochondrial is a two-layer membrane coated organelle in most cells. It is the energy-producing structure of the cell and is the main place for cells to breathe oxygen, known as “power house.” Oxidative phosphorylation of mitochondria provides more than 90% of oxygen consumption and ATP for the whole body. The inactivation of pyruvate dehydrogenase, the reversible inhibition of cytochrome by NO, and the inhibition of mitochondrial respiratory enzyme complex by peroxynitrite are the main factors of cell dysfunction and anoxia. Obviously, in severe systemic inflammatory states, especially sepsis, cellular metabolic changes and organ dysfunction are not only common but predict long-term morbidity and mortality. Clearly, mitochondria is not only the target of intracellular injury but also the reaction site of the body to exogenous stress, which has been the focus of basic science and clinical research. However, mitochondria have many metabolic and signaling functions which may play a central role in sepsis expression and final outcome. Along with bioenergetics, mitochondria participate in several important functions, including calcium flux, program cell death pathway, and redox signaling [38, 39]. The exact reason for mitochondrial dysfunction during sepsis is still unclear. However, inflammatory molecules such as carbon monoxide, nitric oxide (NO), and reactive oxygen/nitrogen species directly damage several components

of the mitochondrial ETC complexes and mitochondrial respiration [40, 41]. Furthermore, lower metabolic rates in sepsis have been related to decreased expression of major components of electron transfer chain (ETC) complexes and the amounts of mitochondrial DNA [42]. This is an important issue because mitochondrial DNA code nearly 80% of mitochondrial protein. Besides decreased amounts of ATP synthase and major components in mitochondrial respiratory chain complexes, recent studies have shown that pyruvate dehydrogenase expression was diminished in sepsis and ARDS [43, 44]. It is important to note that preexisting factors contribute to the severity of sepsis, including environmental exposure to toxins, cigarette smoking, metabolic syndrome of obesity and aging, and diabetes [45, 46]. A clinical analysis of sepsis has shown that the extent of mitochondrial impairment in lungs was significantly correlated with mortality rate. In particular, sepsis-associated mortality is significant in patients that develop acute respiratory distress syndrome (ARDS) [47, 48]. Patients who died from severe sepsis had decreased muscle ATP content, while higher levels of ATP were seen in survivors [49]. Patients who died from severe sepsis had lower levels of muscle ATP, while higher levels of ATP were detected in survivors. Organ dysfunction and clinical illness are associated with decreases in mitochondrial mass and metabolic rate [42]. However, it is possible to restore metabolic activity and organ function and is strongly regulated by expression of markers of mitochondrial biogenesis such as PPAR gamma-coactivator-1a (PGC-1a) and nuclear respiratory factors 1 and 2 (NRF-1 and -2) and via repression of the biogenesis suppressor nuclear receptor interacting protein-140 (RIP140) [50, 51]. Moreover, most recent preclinical studies have shown that not only preservation but also mitochondrial biogenesis is a key in recovering immune or tissue organ homeostasis during sepsis [52, 53].

16.7 Diagnosis

16.7.1 Clinical Signs and Symptoms of Neonatal Sepsis

Neonates with bacterial sepsis might exhibit nonspecific symptoms and symptoms and signs or focal signs of infection, including temperature instability, poor perfusion with pallor and mottled skin, cyanosis, hypotension, apnea, respiratory distress, grunting, tachycardia or bradycardia, metabolic acidosis, irritability, lethargy, seizures, abdominal distention, feeding intolerance, purpura, jaundice, petechiae, and bleeding (Table 16.1). Initial symptoms might be few and could include apnea alone or tachypnea with retractions, grunting, nasal flaring, or tachycardia. Later complications of sepsis might include pulmonary hypertension, respiratory failure, cardiac failure, renal failure, shock, cerebral or thrombosis, liver dysfunction, adrenal hemorrhage or insufficiency, bone marrow dysfunction (anemia, neutropenia, thrombocytopenia), and DIC. The manifestations of noninfectious organ failure may be similar to the clinical manifestations of neonatal sepsis. In addition, both infectious and noninfectious causes may be present at the same time.

Table 16.1 Initial signs and symptoms of infection in newborn infants

Symptoms	
General	Fever, temperature instability; poor feeding, edema or "not doing well"
Gastrointestinal system	Vomiting, diarrhea, abdominal distention, or hepatomegaly
Respiratory system	Apnea, tachypnea, dyspnea, flaring, retractions, grunting, or cyanosis
Renal system	Oliguria
Cardiovascular system	Cold, clammy skin, pallor, mottling, tachycardia, bradycardia, or hypotension
CNS	Irritability, lethargy, tremors, seizures, hypotonia, hyporeflexia, abnormal Moro reflex, full fontanel, irregular respirations, or high-pitched cry
Hematological system	Jaundice, splenomegaly, pallor, petechiae, purpura, or bleeding

Adapted from Nelson Textbook of Pediatrics [54] with permission from Elsevier

16.7.2 Conventional Diagnostics

Traditionally, the diagnosis of neonatal sepsis depends on laboratory culture of isolating the causative agent from a normally sterile body site (blood, urine, CSF, and joint, pleural, and peritoneal fluids; Table 16.2). In order to optimize the diagnosis, it is necessary to obtain aseptic specimens of adequate volume. For blood culture, at least 0.5–1 mL of blood should be obtained, preferably from two different venipuncture sites. The true pathogens are more likely to be present in both cultures. In the presence of central venous catheters, it is best to simultaneously carry out blood cultures, one from a vascular catheter and the other from a peripheral blood, so that differential time to positivity can be assessed [55]. This helps to identify peripheral bacteremia and catheter-related bloodstream infections and is of significance for clinical management. Since certain pathogens may be detected only in cerebrospinal fluid but not in blood, sepsis assessment should also include a lumbar puncture procedure in symptomatic newborns. Automatic blood culture system continuously monitors the specimen and sends out an alarm when the positive signal is detected, thus facilitating the further processing of pathogen identification. Matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry can assist in the early identification of blood cultures and allow pathogen-targeted antibiotic treatment in the case of blood infections [56]. In recent years, multiple PCR techniques have been used to identify common bacteria and fungi, as well as drug-resistant genes in blood culture-positive specimens within a few hours of organism growth. Similar techniques have been used in cerebrospinal fluid samples to shorten the time required for bacterial identification. Urinary tract infections do not occur in the first 72 h of life, so suprapubic bladder aspiration or catheterization is not done as part of the assessment of early neonatal sepsis. However, urinary tract infections are common in term and preterm infants and are an important source of late-onset sepsis in newborns [57]. Pathological examination of placenta may indicate chronic and acute intrauterine inflammation. Although placental cultures can detect

Table 16.2 Culture-based and culture-independent diagnostics for neonatal sepsis (From Andi L Shane, Pablo J Sánchez, Barbara J Stoll. Neonatal sepsis. Lancet, 390, 10104–1770)

	Parameter	Optimal conditions for specimen collection	Applicability for neonatal sepsis
Culture-based			
Blood	Culture	0.5–1 mL of whole blood from two sites at time of symptom onset	Gold standard for bacteremia
CSF	Culture	When clinically feasible, >1 mL CSF	Optimize antimicrobial therapy
Urine	Culture	At >72 h of life, >1 mL urine	Not useful for EOS; potential benefits for LOS
Tracheal aspirate	Culture	Obtained with concern for new onset of lower respiratory tract infection	Usually reflects colonization
Culture-independent			
Immune function	MHC II and TNF-α	Investigational	Both decreased in chorioamnionitis and sepsis
Neutrophil indices	Neutropenia a0bsolute neutrophil count Absolute immature neutrophil count	After 12 h of life, with consideration of gestational age, delivery method, altitude, arterial versus venous blood sampling, and time since birth	Neutropenia better predictor for sepsis than leukocytosis
Neutrophil markers	CD64	Increased for 24 h after infection, requires 50 μL of blood, investigational	Cutoff points between 2.38 and 3.62 optimal sensitivity, specificity, and NPV for EOS
Platelet count	Thrombocytopenia and thrombocytosis	Late findings occurring after clinical manifestations have occurred, usually >72 h after infection onset	Thrombocytopenia associated with fungal infection
CSF cell count	CSF WBC	Uninfected neonates mean 10 cells per mm^3, range up to 20 cells per mm^3	Does not predict culture-proven meningitis
CSF chemistries	CSF protein and glucose concentrations	Full-term <0.1 g/dL, with preterm neonates with higher concentrations (70–80% of serum glucose)	Increased in fungal meningitis; low glucose specific for bacterial meningitis
Acute phase reactant—CRP	CRP	CRP assessed 8–24 h after infection	Good NPV

(continued)

Table 16.2 (continued)

	Parameter	Optimal conditions for specimen collection	Applicability for neonatal sepsis
Acute phase reactant—procalcitonin	Procalcitonin	Procalcitonin assessed 2–12 h after infection, investigational	Better sensitivity but less specificity than CRP
Sepsis panels scores	Multiple laboratory tests	After 24 h of life, investigational	Most useful for NPV and discontinuation of antimicrobial therapy

Adapted from Nelson Textbook of Pediatrics [34] with permission from Elsevier. Routine refers to an assay or parameter that is usually available and widely used. Investigational refers to an assay or parameter that is undergoing assessment for clinical use and applicability
CSF cerebrospinal fluid, *EOS* early-onset sepsis, *LOS* late-onset sepsis, *MHCII* major histocompatibility complex class II, *TNF-α* tumor necrosis factor α, *NPV* negative predictive value, *WBC* white blood cell count, *CRP* C-reactive protein

potentially pathogenic bacteria, these findings do not indicate that the fetus has come into contact with the pathogen, nor is it a true infection, so this should not be a reason for long-term antibiotic therapy in infants.

16.7.3 Culture-Independent Diagnostics

Because PCR is a highly sensitive and rapid technique, it is increasingly being applied to bodily fluids directly without the need to first culture causative agents (Table 16.2). Quantitative real-time amplification systems (qPCR) based on bacterial 16S ribosomal DNA have a very high negative predictive value, and results are usually available in a timely manner. Additionally, a small volume sample is frequently sufficient, and the test can be done on surgical tissues and body fluids such as pleural effusions and ascites. Disadvantages of qPCR include the inability to do susceptibility testing and a high sensitivity that does not differentiate between active infection and recent infections that have resolved [58]. The possibility of detecting contaminants is also high, and therefore clinical correlation with results is mandatory. Other commonly used non-culture-based diagnostic tests include the total and differential white blood cell (WBC) count, absolute and immature neutrophil counts, and the ratio of immature to total neutrophils (I/T). Although the WBC count has limitations in terms of sensitivity, an immature-to-total neutrophil ratio of 0.2 or greater has been suggestive of a bacterial infection. The I/T score was found to be predictive when used in combination with complete blood cell counts obtained at more than 4 h of age [59]. However, abnormal WBC counts could also result from fetal exposure to in utero inflammation and not sepsis as frequently seen following maternal chorioamnionitis. It seems that the main benefit of the WBC count is its negative predictive value since normal serial values make it unlikely that a blood or CSF culture will be positive. It is also worth noting that WBC values are dynamic during the first 12 h of life, so serial measurements over 24 h might be more informative than a single assessment. Other diagnostic tests that measure an

inflammatory response include CRP, procalcitonin (PCT), haptoglobin, fibrinogen, proteomic markers in amniotic fluid, inflammatory cytokines (including IL-6, IL-8, and TNF-α), and cell surface markers (including soluble CD14 subtype [presepsin], and neutrophil CD64) [60, 61]. CRP is commonly used as a marker of bacterial infection. Because of the need to synthesize CRP in the liver before obvious concentrations were found, serum CRP increases within 6–10 h after infection and reached a peak at 2–3 days after infection, resulting in a decrease in sensitivity. Serum PCT increases within 4 h and peaks at 18 and 24 h after onset of the disease. Continuous detection of CRP and other acute phase reactants and markers, such as PCT and interleukin (IL-6) and interleukin (IL-8), may improve the accuracy of the diagnosis of infection [62, 63]. Similar to the WBC count, these nonspecific inflammatory markers have a high negative predictive value if there are no continuous abnormalities, supporting the cessation of antibiotic therapy.

16.7.4 Novel Diagnostic Approaches to Neonatal Sepsis

16.7.4.1 Cord Blood

The cell composition of umbilical cord blood is similar to that of the peripheral blood of fetus during last stage of gestation. It is the first hematologic source from the neonate. It does not require invasive operation nor cause the newborn to feel pain, so the collection of specimens avoids the iatrogenic complications. It also gets more blood, which helps reduce iatrogenic blood loss and does not lead to hemodynamic instability in the newborn. A study analyzing 350 pairs of samples from peripheral venous and umbilical cord samples found a significant correlation between leukocyte and platelet counts (correlation coefficients $r = 0.683$ and $r = 0.54$, respectively) and a lower correlation between hemoglobin ($r = 0.36$) [64]. This may be explained because cord blood from a premature infant may not be the same as the peripheral blood of a premature baby. Although no cases of EOS were detected, the contamination rate of cord blood was higher than that of peripheral blood (12% vs 2.5%) [64]. Another study involving 200 newborns found that cord blood samples had a low contamination rate of just 0.5% [65]. This study confirmed that hematocrit, platelets, WBC, and ANC have similarities between cord and peripheral blood. A study assessing 40 newborns with 2 or more risk factors for EOS found that cord blood had 100% sensitivity and 95% specificity compared to the peripheral blood. However, cord blood CRP was negative in all 11 neonates with positive screening for sepsis [66]. Another review of 15 studies evaluating more than 2000 episodes of suspected neonatal infection identified PCT and IL-6 in cord blood as having high positive (5.72 and 9.47, respectively) and negative likelihood ratios (0.20 and 0.10, respectively) [67].

16.7.4.2 Novel Biomarkers

Proteomics is a new field of research in the post-genetic age. Proteomics is used to isolate and identify differentially expressed proteins in body fluids (mainly blood and urine) from patients with sepsis, which can help to find the biomarkers for the

early diagnosis of sepsis, and has important significance for the early diagnosis, disease monitoring, prognosis evaluation, the pathogenesis of sepsis, and the discovery of drug targets [68]. Based on proteomic analyses, a mass restricted scoring strategy has been devised using relevant proteomic biomarkers. These measurements of amniotic fluid have provided information regarding the fetal response to intra-amniotic inflammation and have successfully predicted EOS with >92% accuracy [69, 70]. On the basis of proteomics analysis, a large-scale restricted scoring strategy was designed by using the related proteomic biomarkers. The proteome analysis of amniotic fluid provided a wealth of information about the fetus's response to intra-amniotic inflammation and successfully predicted EOS with more than 92% accuracy. Altered protein expression patterns have been found through proteomics analysis of cord blood. In a prospective cohort study, a proteomics approach identified biomarkers that were validated and identified two promising biomarkers in proapolipoprotein CII and a des-arginine variant of serum amyloid A [71]. When infants were stratified by risk category based on a score computed using these two concentrations, these markers might play an important role in guiding antimicrobial management decisions and excluded sepsis with 100% negative predictive value [71]. Metabonomics is a newly developed discipline after genomics and proteomics. It is an important part of systems biology. Metabonomics is the study of individual metabolic profiles. The increase of some metabolites can be detected in patients with sepsis, which has a very good diagnostic value [72]. A study found that the combined use of 1H-NMR and GC-MS to analyze the metabolic profile of the urine fluid of newborns can effectively distinguish infection from non-infection and also distinguish between early and late neonatal sepsis [73].

16.8 Management

16.8.1 Fluid Resuscitation

Hypovolemia is common in neonatal sepsis and is associated with poor prognosis. Hypovolemia can be divided into absolute deficiency (loss of blood volume) and relative deficiency (redistribution of blood volume). There is redistribution of blood volume in neonatal sepsis. Fluid resuscitation plays an important role in neonates. The pathophysiological changes of neonatal sepsis are different from those of adults, which are characterized by increased systemic vascular resistance and decreased cardiac output (cold shock). Because the children body surface area is relatively large, the loss of water is relatively more, and the fluid resuscitation treatment can be better tolerance to a little more liquid [74]. Resuscitation volume at 1 h is 10–60 mL/kg to maintain normal perfusion and blood pressure in full-term infants, while liver enlargement and dyspnea should be observed. In preterm infants, there is not enough evidence that a large-volume fluid resuscitation can achieve effects similar to those of full-term infants. For preterm infants with low blood pressure, 10–20 mL/kg saline is recommended within the 30–60 min. This more cautious approach attributed to reports that rapid changes of blood pressure in premature infants of less than 30 weeks could increase the risk of cerebral hemorrhage [75] .

16.8.2 Treatment Against Infectious Agents

16.8.2.1 Therapy for the Suspected Pathogens

The timing of antibiotic therapy for patients with sepsis has been shown to be a key factor in the survival, and several studies have shown that delays in appropriate antibiotic therapy are associated with increased mortality in patients with sepsis and septic shock. It is essential to draw blood for culture before antibiotics are used. But blood culture is of low sensitivity and takes a long time, which is easy to delay treatment. In addition, we should not excessively delay the use of antibiotics in critical neonates with sepsis because of the collection of specimens. In general, empirical treatment should be guided by common antimicrobial resistance patterns of bacterial isolates in community settings or neonatal intensive care units. Ampicillin and third- or fourth-generation cephalosporins are primary empirical treatments for early-onset bacterial infections and can be used in suspected Gram-negative meningitis. Infections caused by extended-spectrum β-lactamase-producing Gram-negative bacilli require treatment with carbapenems, such as meropenem. Treatment with ampicillin–sulbactam and piperacillin–tazobactam is being used increasingly among infants admitted to hospital in the NICU; however, since tazobactam does not easily penetrate into the central nervous system, it should not be used in the treatment of meningitis. However, the β-lactamase inhibitor sulbactam in combination with ampicillin does seem to achieve high concentrations in the CSF (Table 16.3). Although bloodstream infections caused by *coagulase-negative*

Table 16.3 Management and prevention of neonatal sepsis (From Andi L Shane, Pablo J Sánchez, Barbara J Stoll. Neonatal sepsis. Lancet, 390, 10104–1770)

	Therapy	Additional considerations
Empirical management		
EOS	Ampicillin plus aminoglycoside; 10 days for bacteremia; 14 days for GBS bacteremia and uncomplicated meningitis; extend to 21–28 days for complicated infections	Consider a third-generation cephalosporin (cefotaxime preferred) or carbapenem for meningitis; tailor therapy to pathogen; consider discontinuation of therapy if pathogen not isolated
LOS	Vancomycin plus minoglycoside; duration of treatment dependent on pathogen and site	Alternatives to vancomycin can be considered on the basis of local epidemiology and clinical presentation; an aminoglycoside-based regimen is preferred to cephalosporin given the reduced risk of resistance; consider cephalosporin if meningitis is suspected, a carbapenem if the patient has recently been given a third-generation cephalosporin, and amphotericin for fungal causes, and tailor therapy to pathogen, and consider discontinuation of therapy if pathogen is not isolated

(continued)

Table 16.3 (continued)

	Therapy	Additional considerations
Non-antimicrobial treatment strategies		
Recombinant G-CSF and recombinant GM-CSF	Enhance neutrophil number and function, but no reduction in infection when administered as prophylaxis or improvement in survival when administered as therapy	Insufficient evidence to support the clinical use of G-CSF or GM-CSF either as treatment or prophylaxis to prevent systemic infections
IVIG	Augments antibody-dependent cytotoxicity and improves neutrophilic function but no evidence that IVIG in suspected or proven sepsis reduces death	Insufficient evidence from 10 RCTs or quasi-RCTs to support use in neonates with confirmed or suspected sepsis
Prevention strategies		
IAP	Administration of penicillin or ampicillin 4 h before parturition	Successfully reduces rates of EOS due to GBS; and no effect on LOS GBS
Fluconazole prophylaxis	Administration of weight-based dosing to neonates less than 1500 g	Most beneficial in NICUs with high baseline rates of invasive candidiasis
BLF supplementation with a probiotic, LGG	BLF is a human milk glycoprotein with a role in innate immune response. LGG enhances the activity of lactoferrin	BLF supplementation with and without LGG reduced the incidence of first LOS in 472 VLBW neonates in large randomized, double-blind RCT. Additional confirmatory studies warranted

No recommended durations are provided for non-antimicrobial therapies since there is insufficient evidence for their use. Adapted from Nelson Textbook of Pediatrics [34] with permission from Elsevier

EOS early onset sepsis, *GBS* group B streptococcus, *LOS* late-onset sepsis, *IVIG* intravenous immunoglobulin, *RCT* randomized controlled trials, *IAP* intrapartum antimicrobial prophylaxis, *NICU* neonatal intensive care unit, *G-CSF* granulocyte colony stimulating factor, *GM-CSF* granulocyte macrophage stimulating factor, *BLF* bovine lactoferrin supplementation, *LGG* Lactobacillus rhamnosus GG, *VLBW* very low birthweight

staphylococci in premature infants are associated with a high number of short-term morbidity and long-term neurodevelopmental disorders, they are not associated with increased mortality. The use of broad-spectrum antibiotics can cause some additional damages, including overgrowth of potentially pathogenic intestinal flora. The intestinal flora can change in a matter of days. Antibiotics can turn bacteria in the gut or elsewhere into drug-resistant bacteria. So we should always weigh the pros and cons of antibiotics. Long-course antibiotics are often unhelpful and can cause drug-resistant bacteria to overgrow beyond the normal colonization of symbiotic colonies. Fungal infections including candidiasis, zygomycoses, and aspergillosis should be treated actively when they are suspected and diagnosed. Newborns belong to a special group, which is a high incidence of fungal sepsis. In the NICU, newborns belong to a special group, which is a high incidence of fungal sepsis; the

incidence was 1.2% in all hospitalized newborns, 3.1% in very low birth weight, and 5.5% in extremely low birth weight, and the mortality of infected infants was 22.9%. Premature/low birth weight, endotracheal intubation and mechanical ventilation, long duration of antibiotic use, and long hospital stay were risk factors. Empirical antifungal therapy with amphotericin deoxycholate can be feasible for infants at high risk for invasive candidiasis. In order to optimize the use of antimicrobials, a pharmacist with expertise in neonatal infections and a pediatric infectious disease doctor are required to participate in the development of treatment programs. Blood concentrations of antifungal drugs should be monitored to improve the efficacy of drug treatment or reduce toxicity of drugs if these drugs are used for more than 2–3 days. Antibiotics can be safely stopped at 48–72 h in neonates with negative blood cultures who are clinically stable [76]. Around 90% of positive blood cultures grow by 48 h and 97% by 72 h. Most cultures that turn positive after 72 h are contaminants [77]. Discontinuation of antibiotics after the blood culture is reported negative at 48 h in clinically stable newborns and does not increase treatment failure [78]. Continuous CRP detection plays an important role in guiding and shortening the duration of antibiotic therapy [76]. The single and continuous values 24 h after the onset of symptoms had higher negative predictive values (98–100%) [79]. However, a recent study found that the negative predictive value of CRP at 48 h was only 86% [80]. Previous studies have ruled out high-risk babies, such as those with centerline, asphyxia, or mechanical ventilation. The value of CRP in guiding antimicrobial therapy may be limited to a selected population [76]. I/T scores and procalcitonin have also been used to guide treatment with encouraging results, but there's still a need for a large clinical trial [81, 82].

16.8.2.2 Therapy for the Known Pathogens

Once the pathogen of neonatal sepsis is identified, antibiotics that are sensitive to the pathogen should be used as early as possible. Penicillin or ampicillin is effective against GBS and can be discontinued if blood and CSF cultures are sterile. Enterococci should be treated with a penicillin-containing antibiotic, with the addition of an aminoglycoside if synergy is documented to provide bactericidal and post-antibiotic effects. Infections due to ampicillin-resistant enterococci are treated with vancomycin without the addition of an aminoglycoside. Ampicillin alone is effective against L-monocytogenes, although the aminoglycoside also provides synergy at treatment onset. For Gram-negative enteric bacteria, an aminoglycoside or ampicillin (if susceptible) is sufficient for treatment. However, if meningitis is suspected or confirmed, a third-generation or fourth-generation cephalosporin or carbapenem agent should be used. Invasive infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* spp. are best treated with a carbapenem treatment, although cefepime might also be effective. Metronidazole, ampicillin–sulbactam, clindamycin, and metronidazole are effective for anaerobic infections; metronidazole is the first choice for anaerobic infections associated with the CNS. Because most of coagulase-negative staphylococci and MRSA are resistant to β-lactam antibiotics, vancomycin should be used empirically. Teicoplanin, as a new generation glycopeptide antibiotic, is recommended for newborns who

cannot tolerate vancomycin. It has the same efficacy as vancomycin but has less side effects. Amphotericin deoxycholat is still the first choice for invasive candidiasis when meningitis may exist; liposome amphotericin or hydatid (Caspofungin or micafungin) is the best choice for hepatic or splenic candidiasis. Fluconazole may be an effective drug for susceptible organisms. If the infection is associated with central venous catheter access, successful treatment outcomes depend on the duration of positive cultures, underlying condition of the host infant, and ability to remove the source. The exact duration of antimicrobial therapy is not supported by sufficient evidence; however, at a minimum, antibiotics should be discontinued if the cultures are sterile and clinical recovery is evident. It used to be thought that the duration of antibiotic therapy was 7 days for bloodstream infections, 14 days for Gram-positive meningitis, and 21 days for Gram-negative meningitis. However, prolonged use of antibiotics often leads to bacterial resistance, such as the widespread use of vancomycin, which has led to the emergence of vancomycin-insensitive *Staphylococcus aureus* and *vancomycin-resistant Enterococcus*. Interestingly, a study has found that 5 days of ceftriaxone was sufficient to cure bacterial meningitis caused by *Neisseria meningitidis*, *Haemophilus influenzae* type b, and *Streptococcus pneumoniae* [83]. Infants who have been exposed to antibiotics have been shown to have higher rates of necrotizing enterocolitis, sepsis, and morbidity than infants who have not been exposed to antibiotics, presumably due to intestinal dysbiosis induced by antibiotic exposure [84].

In summary, based on our experience, our principles for the use of antibiotics in neonatal sepsis include the following: (1) antibiotics should be used early in the newborns who have highly suspected or proven infection, (2) early discovery of the source of infection is the key to effective treatment, (3) anti-infection and fluid resuscitation should be carried out simultaneously, (4) antibiotics can only kill pathogens but cannot correct immune disorder, and (5) if the evidence of infection does not exist, antibiotics should be discontinued in time.

16.8.3 Restoring Immune Balance

In neonatal sepsis, the innate immune system and the innate immune cells are overactivated, leading to severe and persistent inflammation. A large number of cytokines and inflammatory mediators, such as IL-1, IL-6, IL-17, and TNF-α, were uncontrollably produced in a short period of time. This systemic inflammatory process can result in MODS and death. Therefore, it is important to control the inflammatory response in the early stages of sepsis. However, all RCTs on anti-IL-1, anti-IL-10, anti-PAF, recombinant human soluble thrombomodulin, and recombinant bactericidal permeability-increasing protein (BPI) were negative [84–86]. Some molecules were detrimental, like recombinant tissue factor pathway inhibitor and nitric oxide synthase inhibitors [87]. Recent studies demonstrated that a long-term immunosuppression was a major reason for the high mortality rate in long-term outcomes. Understanding immunodynamics of sepsis

and its immunosuppression is very important to decrease mortality. Hyperinflammation is in the early stage of the immunodynamics of sepsis. The exorbitant release of inflammatory mediator and cytokines impairs tissue cells in inflammatory reaction and, at the same time, also causes immune cells damage and immune suppression consequently, which is the basis of sepsis immunosuppression. Middle stage of sepsis is mixed immune status. During this period, inflammatory and anti-inflammatory mechanism competes with each other, while the inflammatory lesions continue and immune function is further restrained. In the later stage of sepsis, further deterioration and severer immunosuppression leads to immunoparalysis in the end. So far, there is no effective treatment for immunosuppression or immunoparalysis of sepsis. Including glucocorticoid, non-steroid anti-inflammatory drug, TNF-a antibodies, IL-1 antagonists, and so on, a meta-analysis reported that the different types of colony-stimulating factors (CSFs) seem to be ineffective for neonatal sepsis [88], as were granulocyte-CSF (G-CSF) and granulocyte-macrophage CSF (GM-CSF). Immune globulins (IVIG) are effective in adults with sepsis [89] but not in newborns with gestational ages of 31–42 week [90] or low birth weight newborns (<1500 g) [91]. At present, it is now believed that the control of the early inflammatory reaction is the key to preventing immunosuppression or immunoparalysis of sepsis. The main approaches include timely detection and diagnosis of sepsis, rational use of antibiotics, adequate fluid resuscitation, improvement of microcirculation, and so on. Meanwhile, the occurrence of iatrogenic immune disorder should be avoided as far as possible. Extracorporeal blood purification techniques have the functions of clearing toxins and inflammatory factors, stabilizing hemodynamics, regulating liquid balance, and so on. It can also be used in the treatment of sepsis complicated with shock and acute respiratory distress syndrome (ARDS). A meta-analysis of RCTs reported that, overall, hemoperfusion; blood purification techniques, hemofiltration and hemodialysis; and plasma exchange decrease the mortality rate of patients with sepsis (RR0.69, 95%CI, 0.56–0.84). The most positive effect was attributable to two RCTs on plasma exchange (RR 0.63, 95% CI 0.42–0.96) and ten RCTs on polymyxin B hemoperfusion (RR 0.63, 95% CI, 0.50–0.80) [92]. The efficacy of blood purification techniques must be estimated by a large RCT before they can be strongly recommended [93]. The cost/benefit ratio of the technologies for newborns and infants should be fully assessed since the risk of mortality in this population is much lower than in adults. At the same time, it is often difficult because of the need to quickly establish vascular access [94].

16.8.4 Supportive Care

The rapid identification of neonatal sepsis is the key to successful treatment, because the blood pressure in the early stage of neonatal sepsis does not decrease and may even increase. A detailed physical examination that includes normal peripheral pulses, capillary refill of not more than 2 s, urine output of more than

1 mL/kg/h, or normal mental status may be more reliable than blood pressure. In cases of refractory shock, albumin can be given. Hydrocortisone is also an option for the treatment of children with fluid- and catecholamine-resistant shock and suspected or proven adrenal insufficiency, although its efficacy remains to be determined. Blood glucose control and vasopressin are also proposed, but two RCT studies in children have failed to demonstrate any clinical benefit of tight blood glucose control (4.0–7.0 mmol/L versus less than 12.0 mmol/L) [95] and vasopressin [96]. Once the shock is resolved, patients with refractory fluid overload (>10%) can be treated with continuous veno-venous hemofiltration or intermittent hemodialysis. Early goal-directed therapy (EGDT) has been considered as an important method to reduce mortality through the use of invasive monitoring measures to mediate fluid resuscitation in patients with septic shock and has been recommended by international guidelines such as the "Surviving Sepsis Campaign." In recent years, large-scale multicenter studies suggested that EGDT strategy could not reduce the mortality of the patients with severe sepsis and septic shocks and may bring some adverse effects. However, early fluid resuscitation and monitoring is still an important means of treatment of septic shock; therefore, EGDT is still have some value. In children, a RCT study involving 102 Brazilian children with severe sepsis or fluid refractory shock found that the goal and bundle could reduce mortality by 11.8% compared with 39.2% in the control group. AS very few newborns were enrolled in the study; the prevalence of neonatal sepsis would need to be confirmed [97]. Improving oxygen delivery is one of the central goals of supportive care; it is usually achieved by increasing the hemoglobin level. However, RBC transfusions are not completely safe [98]. We do not know the optimal RBC infusion threshold in patients with sepsis, especially for unstable patients. A clinical analysis involving 137 patients with sepsis suggests that restrictive erythrocyte transfusion strategies (hemoglobin threshold values of 7 g/dL) may be safe in stable or stabilized children and neonates with sepsis, even if they are in septic shock [99]. However, RBC transfusion needs in unstable neonates and infants with sepsis still remain unclear.

16.9 Conclusion

Neonatal sepsis has higher morbidity and mortality rate especially in developing countries. The susceptibility of neonates, pathogen variability between different regions, complex pathogenesis, and lack of consensus in the definitions obstruct the development of clinical trials and practice guidelines. Physicians face multiple problems in diagnosis and treatment decisions. Most of them feel stressed enough to treat every newborn with suspected sepsis. Therefore many newborns receive prolonged antibiotic therapies, which often lead to many new complications. In fact, comprehensive preventive strategies are very important, including hand hygiene, early breastfeeding, reducing unnecessary operations, the limitation of indwelling devices, etc. Beyond that, we should follow the pathogenesis of neonatal sepsis and develop individualized treatment plan in order to a good outcome.

References

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–10.
2. Wynn JL. Defining neonatal sepsis. Curr Opin Pediatr. 2016;28(2):135–40.
3. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151–61.
4. Brocklehurst P, Farrell B, King A, Juszczak E, Darlow B, Haque K, et al. Treatment of neonatal sepsis with intravenous immune globulin. N Engl J Med. 2011;365(13):1201–11.
5. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126(3):443–56.
6. Cohen-Wolkowiez M, Moran C, Benjamin DK, Cotten CM, Clark RH, Benjamin DK Jr, et al. Early and late onset sepsis in late preterm infants. Pediatr Infect Dis J. 2009;28(12):1052–6.
7. Barton L, Hodgman JE, Pavlova Z. Causes of death in the extremely low birth weight infant. Pediatrics. 1999;103(2):446–51.
8. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA. 2004;292(19):2357–65.
9. Jan AI, Ramanathan R, Cayabyab RG. Chorioamnionitis and management of asymptomatic infants ≥35 weeks without empiric antibiotics. Pediatrics. 2017;140(1):e20162744.
10. Verani JR, McGee L, Schrag SJ, Division of Bacterial Diseases NCfIaRD, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease–revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59:1–36.
11. Kaufman DA, Coggins SA, Zanelli SA, Weitkamp JH. Congenitalcutaneous candidiasis: rompt systemic treatment is associated withimproved outcomes in neonates. Clin Infect Dis. 2017;64(10):1387–95.
12. Barton M, Shen A, O'Brien K, Robinson JL, Davies HD, Simpson K, et al. Early onset invasive candidiasis in extremely low birthweight infants: perinatal acquisition predicts poor outcome. Clin Infect Dis. 2017;64(7):921–7.
13. Vergnano S, Menson E, Smith Z, Kennea N, Embleton N, Clarke P, et al. Characteristics of invasive Staphylococcus aureus in United Kingdom Neonatal Units. Pediatr Infect Dis J. 2011;30:850–4.
14. Investigators of the Delhi Neonatal Infection Study (DeNIS)collaboration. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. Lancet Glob Health. 2016;4:e752–60.
15. Kimberlin DW, Whitley RJ, Wan W, Powell DA, Storch G, Ahmed A, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. N Engl J Med. 2011;365:1284–92.
16. Thompson C, Whitley R. Neonatal herpes simplex virus infections: where are we now? Adv Exp Med Biol. 2011;697:221–30.
17. Verboon-Maciolek MA, Krediet TG, Gerards LJ, de Vries LS, Groenendaal F, van Loon AM. Severe neonatal parechovirus infection and similarity with enterovirus infection. Pediatr Infect Dis J. 2008;27:241–5.
18. Trofa D, Gácser A, Nosanchuk JD. Candida parapsilosis, anemerging fungal pathogen. Clin Microbiol Rev. 2008;21:606–25.
19. Benjamin DK Jr, Stoll BJ, Gantz MG, Walsh MC, Sánchez PJ, Das A, et al. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. Pediatrics. 2010;126:e865–73.
20. Chan GJ, Lee ACC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med. 2013;10:e1001502.
21. Jiang Z, Ye GY. 1:4 matched case-control study on influential factor of early onset neonatal sepsis. Eur Rev Med Pharmacol Sci. 2013;17:2460e6.

22. Mukhopadhyay S, Puopolo KM. Risk assessment in neonatal early onset sepsis. Semin Perinatol. 2012;36:408e15.
23. Weston EJ, Pondo T, Lewis MM, Martell-Cleary P, Morin C, Jewell B, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005–2008. Pediatr Infect Dis J. 2011;30:937–41.
24. Schuchat A, Zywicki SS, Dinsmoor MJ, Mercer B, Romaguera J, O'Sullivan MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. Pediatrics. 2000;105(1 Pt 1):21–6.
25. Wynn JL, Levy O. Role of innate host defenses in susceptibility to early-onset neonatal sepsis. Clin Perinatol. 2010;37:307e37.
26. Stoll BJ, Hansen NI, Higgins RD, Fanaroff AA, Duara S, Goldberg R, et al. Very low birth weight preterm infants with early onset neonatal sepsis: the predominance of gram-negative infections continues in the National Institute of Child Health and Human Development Neonatal Research Network, 2002–2003. Pediatr Infect Dis J. 2005;24:635–9.
27. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110:285–91.
28. Samuelsson A, Isaksson B, Hanberger H, Olhager E. Late-onset neonatal sepsis, risk factors and interventions: an analysis of recurrent outbreaks of Serratia marcescens, 2006–2011. J Hosp Infect. 2014;86:57–63.
29. Hoffman MA, Snowden JN, Simonsen KA, Nenninger TM, Lyden ER, Anderson-Berry AL. Neonatal late-onset sepsis following peripherally inserted central catheter removal: association with antibiotic use and adverse line events. J Infus Nurs. 2015;38:129–34.
30. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.
31. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124(4):783–801.
32. de Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002;166(1):98–104.
33. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, et al. EarlyMicrocirculatory perfusion derangements in patients with severe sepsis and septic shock:Relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88–98, 98 e81-82
34. Sakr Y, Dubois MJ, de Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med. 2004;32(9):1825–31.
35. Ince C. The microcirculation is the motor of sepsis. Crit Care. 2005;9(Suppl 4):S13–9.
36. Spronk PE, Zandstra DF, Ince C. Bench-to-bedside review: sepsis is a disease of the microcirculation. Crit Care. 2004;8(6):462–8.
37. Ait-Oufella H, Maury E, Lehoux S, Guidet B, Offenstadt G. The endothelium: physiological functions and role in microcirculatory failure during severe sepsis. Intensive Care Med. 2010;36(8):1286–98.
38. Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. Cell. 2006;125:1241–52.
39. Osellame LD, Blacker TS, Duchen MR. Cellular and molecular mechanisms of mitochondrial function. Best Pract Res Clin Endocrinol Metab. 2012;26:711–23.
40. Singer M. Mitochondrial function in sepsis: acute phase versus multiple organ failure. Crit Care Med. 2007;35(9 Suppl):S441–8.
41. Larsen FJ, Schiffer TA, Weitzberg E, Lundberg JO. Regulation of mitochondrial function and energetics by reactive nitrogen oxides. Free Radic Biol Med. 2012;53:1919–28.
42. Haden DW, Suliman HB, Carraway MS, Welty-Wolf KE, Ali AS, Shitara H, et al. Mitochondrial biogenesis restores oxidative metabolism during Staphylococcus aureus sepsis. Am J Respir Crit Care Med. 2007;176:768–77.

43. Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. Virulence. 2014;5:66–72.
44. Carré JE, Orban JC, Re L, Felsmann K, Iffert W, Bauer M, et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med. 2010;182:745–51.
45. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. Virulence. 2014;5:4–11.
46. Ferro TN, Goslar PW, Romanovsky AA, Petersen SR. Smoking in trauma patients: the effects on the incidenceof sepsis, respiratory failure, organ failure, and mortality. J Trauma. 2010;69:308–12.
47. Huttunen R, Laine J, Lumio J, Vuento R, Syrjänen J. Obesity and smoking are factors associated with poor prognosis in patients with bacteraemia. BMC Infect Dis. 2007;7:13.
48. Boulos M, Astiz ME, Barua RS, Osman M. Impaired mitochondrial function induced by serum from septic shockpatients is attenuated by inhibition of nitric oxide synthase and poly(ADP-ribose) synthase. Crit Care Med. 2003;31:353–8.
49. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet. 2002;360:219–23.
50. Chen Y, Wang Y, Chen J, Chen X, Cao W, Chen S, et al. Roles of transcriptional corepressor RIP140 and coactivator PGC-1alpha in energy state of chronically infarcted rat hearts and mitochondrial function of cardiomyocytes. Mol Cell Endocrinol. 2012;362:11–8.
51. Finck BN, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) regulatory cascade in cardiac physiology and disease. Circulation. 2007;115:2540–8.
52. Grégoire M, Tadié JM, Uhel F, Gacouin A, Piau C, Bone N, et al. Frontline science: HMGB1 induces neutrophil dysfunction in experimental sepsisand in patients who survive septic shock. J Leukoc Biol. 2017;101(6):1281–7.
53. Liu Z, Bone N, Jiang S, Park DW, Tadie JM, Deshane J, et al. AMP-activated protein kinase and Glycogen Synthase Kinase 3β modulate the severity of sepsis-induced lung injury. Mol Med. 2015;21:937. https://doi.org/10.2119/molmed.2015.00198.
54. Stoll BJ, Shame AL. Infections of the neonatal infant. In: Kliegman R, Stanton B, St Geme J, Schor N, editors. Nelson textbook of pediatrics. 20th ed. Philadelphia: Elsevier; 2015. p. 909–25.
55. WHO. WHO guidelines on drawing blood: best practices in phlebotomy. 2010. http://apps.who.int/iris/bitstream/10665/44294/1/9789241599221_eng.pdf. Accessed 18 Apr 2017.
56. Malcolmson C, Ng K, Hughes S, Kissoon N, Schina J, Tilley PA, et al. Impact of matrix-assisted laser desorption and ionization time-of-flight and antimicrobial stewardship intervention on treatment of bloodstream infections in hospitalized children. J Pediatric Infect Dis Soc. 2017;6(2):178–86.
57. Ruangkit C, Satpute A, Vogt BA, Hoyen C, Viswanathan S. Incidence and risk factors of urinary tract infection in very low birth weight infants. J Neonatal Perinatal Med. 2016;9:83–90.
58. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol. 2010;37:421–38.
59. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. Pediatr Infect Dis J. 2014;33:798–802.
60. Pugni L, Pietrasanta C, Milani S, Vener C, Ronchi A, Falbo M, et al. Presepsin (soluble CD14 subtype): reference ranges of a new sepsis marker in term and preterm neonates. PLoS One. 2015;10(12):e014602070.
61. Mussap M, Puxeddu E, Puddu M, Ottonello G, Coghe F, Comite P, et al. Soluble CD14 subtype (sCD14-ST) presepsin in premature and full term critically ill newborns with sepsis and SIRS. Clin Chim Acta. 2015;451:65–70.
62. Hofer N, Zacharias E, Muller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology. 2012;102:25–36.
63. Bhandari V. Effective biomarkers for diagnosis of neonatal sepsis. J Pediatric Infect Dis Soc. 2014;3:234–45.

64. Rotshenker-Olshinka K, Shinwell ES, Juster-Reicher A, Rosin I, Flidel-Rimon O. Comparison of hematologic indices and markers of infection in umbilical cord and neonatal blood. J Matern Fetal Neonatal Med. 2014;27:625–8.
65. Beeram MR, Loughran C, Cipriani C, Govande V. Utilization of umbilical cord blood for the evaluation of group B streptococcal sepsis screening. Clin Pediatr (Phila). 2012;51:447–53.
66. Meena J, Charles MV, Ali A, Ramakrishnan S, Gosh S, Seetha KS. Utility of cord blood culture in early onset neonatal sepsis. Australas Med J. 2015;8:263–7.
67. Su H, Chang SS, Han CM, et al. Inflammatory markers in cord blood or maternal serum for early detection of neonatal sepsis-a systemic review and meta-analysis. J Perinatol. 2014;34:268–74.
68. Howman RA, Charles AK, Jacques A, Doherty DA, Simmer K, Strunk T, et al. Inflammatory and haematological markers in the maternal, umbilical cord and infant circulation in histological chorioamnionitis. PLoS One. 2012;7:e51836.
69. Buhimschi CS, Bhandari V, Han YW, Dulay AT, Baumbusch MA, Madri JA, et al. Using proteomics in perinatal and neonatal sepsis: hopes and challenges for the future. Curr Opin Infect Dis. 2009;22:235–43.
70. Buhimschi CS, Bhandari V, Hamar BD, Bahtiyar MO, Zhao G, Sfakianaki AK, et al. Proteomic profiling of the amniotic fluid to detect inflammation, infection, and neonatal sepsis. PLoS Med. 2007;4:e18.
71. Ng PC, Ang IL, Chiu RW, Li K, Lam HS, Wong RP, Chui KM, et al. Host-response biomarkers for diagnosis of late-onset septicemia and necrotizing enterocolitis in preterm infants. J Clin Invest. 2010;120:2989–3000.
72. Dessì A, Corsello G, Stronati M, Gazzolo D, Caboni P, Carboni R, et al. New diagnostic possibilities in systemic neonatal infections: metabolomics. Early Hum Dev. 2014;90(Suppl 1):S19–21.
73. Fanos V, Caboni P, Corsello G, Stronati M, Gazzolo D. Urinary 1 H-NMR and GC-MS metabolomics predicts early and late onset neonatal sepsis. Early Hum Dev. 2014;90:S78–83.
74. Mckiernan CA, Lieberman SA. Circulatory shock in children: an overview. Pediatr Rev. 2005;26(12):451–60.
75. Caresta E, Papoff P, Valentini SB, Mancuso M, Cicchetti R. What's new in the treatment of neonatal shock. J Matern Fetal Neonatal Med. 2011;24(sup1):17–9.
76. Sivanandan S, Soraisham AS, Swarnam K. Choice and duration of antimicrobial therapy for neonatal sepsis and meningitis. Int J Pediatr. 2011;2011:712150.
77. Garciaprats JA, Cooper TR, Schneider VF, Stager CE, Hansen TN. Rapid detection of microorganisms in blood cultures of newborn infants utilizing an automated blood culture system. Pediatrics. 2000;105(3 Pt 1):523–7.
78. Saini SS, Dutta S, Ray P, Narang A. Short course versus 7-day course of intravenous antibiotics for probable neonatal septicemia: a pilot, open-label, randomized controlled trial. Indian Pediatr. 2011;48:19–24.
79. Ehl S, Gering B, Bartmann P, Högel J, Pohlandt F. C-reactive protein is a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection. Pediatrics. 1997;99:216–21.
80. Al-Zwaini EJ. C-reactive protein: a useful marker for guiding duration of antibiotic therapy in suspected neonatal septicaemia? East Mediterr Health J. 2009;15:269–75.
81. Stocker M, Fontana M, El Helou S, et al. Use of procalcitonin-guided decision-making to shorten antibiotic therapy in suspected neonatal early-onset sepsis: prospective randomized intervention trial. Neonatology. 2010;97:165–74.
82. Murphy K, Weiner J. Use of leukocyte counts in evaluation of early-onset neonatal sepsis. Pediatr Infect Dis J. 2012;31:16–9.
83. Molyneux E, Nizami SQ, Saha S, Huu KT, Azam M, Bhutta ZA, Zaki R, Weber MW, Qazi SA. 5 versus 10 days of treatmentwith ceftriaxone for bacterial meningitis in children: a double-blind randomised equivalence study. Lancet. 2011;377(9780):1837–45.
84. Greenwood C, Morrow AL, Lagomarcino AJ, Altaye M, Taft DH, Yu Z, et al. Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of Enterobacter. J Pediatr. 2014;165:23–9.

85. Vincent JL, Ramesh MK, Ernest D, LaRosa SP, Pachl J, Aikawa N, et al. A randomized, double-blind, placebo-controlled, Phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Crit Care Med. 2013;41:2069–79.
86. Levin M, Quint PA, Goldstein B, Barton P, Bradley JS, Shemie SD, et al. Recombinant bactericidal/permeability-increasing protein (rBPI 21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. Lancet. 2000;356:961–7.
87. López A, Lorente JA, Steingrub J, Bakker J, McLuckie A, Willatts S, et al. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival inpatients with septic shock. Crit Care Med. 2004;32:21–30.
88. Carr R, Modi N, Doré CJ. G-CSF and GM-CSF for treating or preventing neonatal infections. Cochrane Libr. 2003;3(3):CD003066.
89. Kreymann KG, de Heer G, Nierhaus A, Kluge S. Use of polyclonal immunoglobulins as adjunctive therapy for sepsis or septic shock. Crit Care Med. 2007;35:2677–85.
90. Akdag A, Dilmen U, Haque K, Dilli D, Erdeve O, Goekmen T. Role of pentoxifylline and/or IgM-enriched intravenous immunoglobulin in the management of neonatal sepsis. Am J Perinatol. 2014;31(10):905–12.
91. The INIS Collaborative Group. Treatment of neonatal sepsis with intravenous immune globulin. N Engl J Med. 2011;365:1201–11.
92. Zhou F, Peng Z, Murugan R, Kellum JA. Blood purification and mortality in sepsis: a meta-analysis of randomized trials. Crit Care Med. 2013;41:2209–20.
93. Kalil AC, Florescu MC. Blood purification: can we purify our patients from sepsis. Crit Care Med. 2013;41:2244–5.
94. Nguyen TC, Kiss JE, Goldman JR, Carcillo JA. The role of plasmapheresis in critical illness. Crit Care Clin. 2012;28:453–68.
95. Macrae D, Grieve R, Allen E, Sadique Z, Morris K, Pappachan J, et al. CHiP investigators: a rando-mized trial of hyperglycemic control in pediatric intensive care. N Engl J Med. 2014;370:107–18.
96. Choong K, Bohn D, Fraser DD, Gaboury I, Hutchison JS, Joffe AR, Canadian Critical Care Trials Group, et al. Vasopressin in pediatric vasodilatory shock: a multicenter randomized controlled trial. Am J Respir Crit Care Med. 2009;180:632–9.
97. de Oliveira CF, de Oliveira DS, Gottschald AF, Moura JD, Costa GA, Ventura AC, et al. ACCM/PALS haemodynamic support guidelines for paediatric septic shock: an outcomes comparison with and without monitoring central venous oxygen saturation. Intensive Care Med. 2008;34:1065–75.
98. Chapman CE, Stainsby D, Jones H, Love E, Massey E, Win N, Serious Hazards of Transfusion Steering Group, et al. Ten years of hemovigilance reports of transfusion-related acute lung injury in the United Kingdom and the impact of preferential use of male donor plasma. Transfusion. 2009;49:440–52.
99. Karam O, Tucci M, Ducruet T, Hume H, Lacroix J, Gauvin F, Canadian Critical Care Trials Group, The PALISI Network. Red blood cell transfusion thresholds in pediatric septic patients. Pediatr Crit Care Med. 2011;12:512–8.

Advances in Stem Cell Research in Sepsis

17

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Abstract

Sepsis is one of the leading causes of death among hospitalized patients despite appropriate antimicrobial and resuscitative approaches. Recent research has focused on exogenous stem cell-based therapy. This chapter is mainly divided into two sections, endogenous stem cell- and exogenous mesenchymal stem cell-based treatment in sepsis. Sepsis-related endogenous stem cells include muscle stem cell dysfunction and hematopoietic stem cell exhaustion and myelosuppression in sepsis. Mesenchymal stem cells (MSCs) display multiple beneficial properties as a promising candidate for stem cell-based therapy by their intrinsic ability to decrease apoptosis and home to injured tissue, beneficially modulate immune cells, secrete paracrine signals (e.g., IL-10, PGE2, and IDO) to limit systemic and local inflammation, activate resident stem cells, and stimulate neoangiogenesis. These effects are associated with improved survival and reduced organ dysfunction in animal models. Indeed, research utilizing sepsis animal

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models have revealed the ability of MSCs to markedly inhibit the multi-organ dysfunction as well as the inciting inflammatory phase of sepsis observed in the later phase of sepsis resulting in improving organ function and survival, and recently clinical trials have demonstrated the safety of intravenous infusion MSCs with sepsis patients. Overall, more multicenter random clinical trials need to be developed to evaluate the role of MSCs and establish the standardized clinical guideline in septic patient in the future.

Keywords
Sepsis · Mesenchymal stem cells · Stem cell-based therapy · Animal sepsis models

17.1 Introduction

Sepsis is a systemic inflammatory response syndrome caused by various microorganisms and immunogenic substances of severe infection and trauma and leads to a secondary injury of tissues and organs. It is a most common complication of serious burning and trauma, shock, infection, and surgery. The further development of sepsis can lead to multiple organ dysfunction syndromes (MODS) and septic shock, which is one of the most important causes of death in clinical critically ill patients. According to the Global Sepsis Alliance, sepsis is the preventable number one cause of death which affects more than 30 million people a year, of whom 6 six to 8 million die. Surviving patients suffer from the consequences for the rest of their lives. Even with appropriate resuscitative and antibiotic therapies, sepsis carries a significant morbidity associated with organ failure and 30% mortality rate [1, 2]. Sepsis incurs a staggering $16.7 billion cost in the US health economy with over 750,000 annual cases and >200,000 deaths each year [3]. Therefore, the search for more effective new therapies has become an important scientific problem urgently needed to be solved in medical research.

Many evidences have elucidated sepsis pathophysiologic mechanisms [3]. Invading pathogen components such as lipopolysaccharide (LPS) activate host immune cell Toll-like receptors (TLRs) to induce a proinflammatory cytokine phase characterized by systemically elevated chemokines, interleukins (IL), and cell adhesion molecules for cellular trafficking between endothelial and immune cell. In sepsis, excessive inflammatory response activates a number of signaling pathways in several types of tissue cell and leads to a secondary injury of tissues and organs including the lung, liver, intestine, muscle, etc. In addition, the repair and regeneration of these secondary injuries of tissues and organs also appears disorders, which is partly associated with the dysfunction of endogenous stem cell in sepsis [4].

Furthermore, recent evidences have demonstrated the exogenous mesenchymal stem cell-based therapy significantly improved the mortality and morbidity resulting from sepsis and deficiencies of our current therapeutic regimens. Stem cells are self-renewing and undifferentiated precursors that retain the capacity for

differentiation into multiple cell types and provide an unending supply of healthy cellular units for damaged tissue. An ever-growing variety of stem cell types have been described to date, and a number of phase I, II, and III clinical trials have been performed to evaluate their clinical efficacy in a variety of human diseases [5]. While it was initially believed that the greatest potential of stem cells resided in their ability to engraft and transdifferentiate to the cell types of injured tissues, it has now been well confirmed that they also exhibit a range of beneficial abilities, including downregulating the inflammatory cascade, modulating the activity of multiple immune cell types, homing to sites of injury, preventing apoptosis in threatened tissues, promoting neoangiogenesis, and activating resident stem cells [6–10]. These features make stem cells an extremely promising therapy for both the proinflammatory response of early sepsis and the widespread organ dysfunction which develops thereafter.

This article will focus upon expanding the understanding of the roles of endogenous stem cell in the development and prognosis of sepsis. Furthermore, this article will also evaluate the functions of exogenous MSCs and examine how the new information provided by recent animal and clinical trials support exogenous MSC-based therapy for sepsis.

17.2 Endogenous Stem Cells

Stem cells are an important resource for tissue repair and regeneration. Normally, stem cell usually resides in the bone marrow and perivascular niches, however, all types of stem cells are attracted from the surrounding tissue, bone marrow, and/or the circulation to serve as progenitor cells, and migrates to the injured tissue area, to repair and regenerate the injured tissue and organs through differentiating into all types of tissue cells, or secreting growth factors to induce the proliferation and growth of local tissue cells. However, the function of stem cells is disorder in the microenvironment in some diseases, which leads to tissue repair and regeneration dysfunction and affects the development and prognosis of disease.

17.2.1 ICU-Acquired Muscle Weakness Associated with Muscle Stem Cell Dysfunction

Normally, skeletal muscle is capable of remarkable regeneration post-injury or trauma, a property endowed by the presence of muscle stem cells and satellite cells (SCs). However, survival sepsis patients frequently suffer from muscle wasting. Critical illness myopathy is a major complication of sepsis in the intensive care unit (ICU). Critical illness myopathy affects up to 4% of sepsis patients and is independently associated with failure of weaning from mechanical ventilation, long-term disability, and increased ICU and post-ICU mortality [11]. The pathophysiology of critical illness myopathy is multifactorial and complex. In addition, the muscle weakness, fatigue, and wasting can persist for up to 5 years, with a remarkable

impact on functional status and quality of life. This outcome suggests an impairment of muscle regeneration conferred by SCs.

Indeed, an early and long-lasting dysfunction of satellite cells that persistently impairs muscle regeneration, replacing a fully functional muscle with a huge fibrotic area, was observed in a validated model of injury and CLP septic mice [4]. As early as 6 h after sepsis, a massive loss of activated/proliferating SCs was observed. Furthermore, the remaining SCs displayed abnormal mitochondrial activity together with loss of mitochondrial mass and degraded mtDNA, but they also displayed hyper-transcription-active, hyper-replication-active, and hyperpolarized organelles leading to an increase of OXPHOS. This impairment was recapitulated in vitro with the inability of SCs to self-renew, divide, and differentiate in an environment containing serum from a sepsis mouse. Three months after sepsis, the muscle was essentially unable to regenerate, which suggests a durable impairment that resembles the impairment observed in the patients that suffer from muscle force loss for up to 5 years after sepsis. In addition, MSC engraftment improves the septic status by restoring mitochondrial and metabolic function in satellite cells, decreasing cytokine levels, and improving muscle strength. These findings indicate that sepsis affects quiescent muscle stem cells and that MSCs might act as a preventive therapeutic approach for sepsis-related morbidity.

17.2.2 Hematopoietic Stem Cell Exhaustion and Myelosuppression in Sepsis

Besides non-hematopoietic stem cell, sepsis also induces hematopoietic stem cell myelosuppression and exhaustion [12]. In the adult, the bone marrow is the central organ for blood production, generating a great number of mature circulating cells daily from a small amount of hematopoietic stem cells (HSC). During bacterial infection, bone marrow hematopoietic stem cells are challenged with the need of expanding progenitor cell pools to recruit the mature immune cells required to fight the pathogens, in particular neutrophils. Sepsis is one of the most dramatic examples of inadequate host bone marrow response to infection; therefore an initial neutrophilia and hyper-reactive immune response is followed by profound leukocyte hyporesponsiveness, neutropenia, and consequently an inability of the host to control the bacterial infection [13].

HSCs act as a direct pathogen stress sensor via activation of Toll-like receptor 4 (TLR4) [14]. However, evidences show that HSCs undergo dysfunctional expansion in the bone marrow in sepsis, which is associated with neutropenia and a block of myeloid differentiation in a TLR4-dependent manner. Furthermore, acute exposure of hematopoietic stem cell to LPS permanently affects their ability to self-renew and engraft. Chronic activation of TLR4 also impairs hematopoietic stem cell functions [15]. All together, these manifest a broad role of TLR4 in the regulation of hematopoietic homeostasis under septic stress conditions.

As we known, TLR4 recognizes LPS, a component of gram-negative bacteria such as *Escherichia coli*, *P. aeruginosa*, and *Salmonella*, which account for 60% of

sepsis cases. Activation of TLR4 induced by its ligand LPS sets off intracellular signaling through two different adaptors: TIR-domain-containing adapter-inducing interferon b (TRIF) and myeloid differentiation factor 88 (MYD88). More evidences confirm that TRIF mediates persistent injury to HSC functions, whereas MYD88 plays a dominant role in myelosuppression. Thus, distinct mechanisms downstream of TLR4 signaling mediate HSC exhaustion and myelosuppression during sepsis through unique effects of TRIF and MyD88 [12]. These results provide a guide to further dissect the independent effects of TRIF and MYD88 during response to severe bacterial infection and support the rationale for pursuing time-tuned selective silencing of one pathway (TRIF or MYD88) to mitigate stem cell injury or myelosuppression.

17.3 Treatment of Exogenous Mesenchymal Stem Cells in Sepsis

Mesenchymal stem cell (MSC) is a kind of precursor cells with self-renewal and differentiation potential distributed in adult perivascular niches throughout the body. De Miguel et al. [16] found that MSC has not only own a strong self-renewal ability and multidirectional differentiation potential into ectodermal, mesodermal, and endodermal lineages but also has a good immune regulation and strong anti-inflammatory properties which affect the immune cell activity and proliferation, differentiation, immune molecular secretion and function, and inhibition of inflammation cascade; inhibit apoptosis; promote angiogenesis; and activate in situ stem cells. Therefore, MSC transplantation may be a promising therapy for sepsis patients by restoring their unbalanced immune system and repairing the injured tissues. With the development of stem cell research, MSCs gradually attract people's attention. MSCs have been shown to prevent the loss of threatened tissues, promote regeneration of injured tissue, and improve overall tissue function following insults such as bacterial infection or ischemia. However, in rapidly evolving diseases such as sepsis, the interaction of MSCs with neighboring cells may assume a greater importance in determining host survival.

17.3.1 Mesenchymal Stem Cell Homing in Sepsis

A great number of studies have confirmed that cell-to-cell dependent and cell-to-cell independent are currently two main functional mechanisms of MSC [17]. In addition, other studies found that MSCs own "homing" capacity which means selective migrate and repair of damaged tissue after transplantation. A recent study confirmed that intravenously administered MSCs migrated with greatest affinity to the liver and lung after LPS-induced sepsis [18], while another study showed the greatest concentration of mesenchymal stem cells in the lung, spleen, and kidney in response to polymicrobial sepsis [19]. This ability to home to diverse injured tissues makes mesenchymal stem cells particularly appealing in the treatment of the

multi-organ dysfunction observed in sepsis. In vitro studies have shown that the homing of mesenchymal stem cells shares many similarities with leukocyte tracking, including chemotaxis, adhesion, and exudation [20].

Several studies have identified a variety of cell surface adhesion molecules expressed by mesenchymal stem cells [21–23]. One of the most important adhesion molecules is vascular cell adhesion molecule (VCAM)-1, which has been shown to be upregulated in mesenchymal stem cells after exposure to tumor necrosis factor-a [22]. It is believed the multi-organ dysfunction in sepsis is related to end-organ hypoxia secondary to the complex coagulation and vasodilatory aberrations known to occur. Ceradini et al. demonstrated that hypoxic tissue production of HIF-1 was associated with an SDF-1 gradient, which correlated with the degree of hypoxia; thus, mesenchymal stem cell homing occurs along this HIF-1-mediated SDF-1 gradient [7]. Ringe et al. also corroborated that the MSCs home to the injured tissues along an SDF-1 gradient [24]. Several in vitro studies have also confirmed that the capacity of mesenchymal stem cells to transmigrate through the extracellular matrix is dependent upon an inflammatory cytokine-mediated MSC modulation of tissue inhibitors of matrix metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs) [25–27].

Several studies have implicated the expression of an array of other MSC chemokine receptors. It was demonstrated that CCR1, CCR4, CCR7, CCR10, and CXCR5 were also involved in MSC homing [28]. Ringe et al. determined that MSCs express CXCR1, CXCR2, CXCR3, CCR2, and CCR8 [24]. A more recent study included CCR5 and CCR9 in the homing of MSCs to inflamed tissues after infusion [29]. The variable range of chemokine receptor expression may control the capacity of mesenchymal stem cells to home to the injured tissues in sepsis. Pretreatment and genetic modifications of MSCs to affect expression of chemokine receptors are currently a hot area of research to improve capacity for MSC homing [30, 31] and may provide better benefits to the efficacy of MSCs in the cell-based therapy of sepsis in the future.

17.3.2 Mesenchymal Stem Cell Therapeutic Effect in Sepsis

Xu and his team firstly reported that MSC transplantation relieved endotoxin-induced lung injury and decreased serum proinflammatory factor levels, thereby reducing lung injury via tail vein injection of mouse for the treatment of sepsis [9]. Li et al. demonstrated that early mortality and organ function of sepsis can be significantly improved, and also late mortality is significantly reduced under MSC treatment [32]. It is believed that MSCs have two functions; one is regulating the inflammatory response, and the other one is increasing the pathogen clearance. Nemeth demonstrated that MSCs can increase the survival rate of sepsis mice by regulating both local and systemic immune environments [20]. They found that the bacterial cell lipopolysaccharide (LPS) combined with TNF-α, which activate downstream NF-κB pathway, promotes the release of cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE-2) by MSC reversed macrophage phenotype to

anti-inflammatory macrophage M2 which reduced the level of TNF-α and IL-6 and increased the level of IL-10, in the sepsis animal model [20]. It is also suggested that one possible mechanism for MSCs to treat sepsis is to inhibit neutrophil migration and reduce the release of neutrophil myeloperoxidase [20]. Luo found that compared with untreated MSCs, IL-1β and TGF-β1 pretreated MSCs could significantly improve cardiac function caused by acute ischemia through the promotion of VEGF secretion to improve myocardial ischemia [33]. In addition, MSCs can directly secrete antimicrobial peptide LL-37 to inhibit the growth of bacteria and reduce bacterial load [34]. Kim JS found that intravenous delivery of MSCs can not only increase the survival rate of mice but also accelerate the lung and spleen bacterial clearance in the treatment of *Mycobacterium tuberculosis* abscess [35]. In addition, MSCs could increase the levels of IFN-γ, TNF-α, IL-6, MCP-1, NO, and PGE-2 and enhance the activity of CD11 high macrophages and increase mononuclear cells recruited to the lungs of mice infected with *Mycobacterium tuberculosis*. MSCs could significantly increase the level of NO by activating NF-κB in macrophages infected with *Mycobacterium tuberculosis* in vitro, and the inhibition of NF-κB activity or inhibition of NO could lead to the decrease of antibacterial activity of MSCs.

Through the above study, we can easily figure that MSCs have shown significant therapeutic effect in inflammation disease especially sepsis. MSCs are more like a "regulator" in the inflammatory response process. The inflammatory tissue is responsible for signaling that MSCs achieve the following functions, including bacterial clearance, inflammation suppression, anti-apoptosis, and cell regeneration. It can not only help the body to resist the inflammation of "cascade" inflammatory cascade in sepsis but also promote the inflammatory reaction in the later period when the immune paralysis occurs and play a role of phagocyte cells clearing the pathogen. All in all, MSCs provide benefit through (1) anti-apoptotic effects, (2) anti-inflammatory effects, (3) immunomodulatory effects on various immune cells, (4) activation of resident stem cells, and (5) neoangiogenesis [6, 19, 36–39]. The preclinical studies data of MSCs in sepsis are shown in Table 17.1.

Recently, Cellular Immunotherapy for Septic Shock (CISS) study (NCT02421484) reported that infusion of freshly cultured allogenic bone marrow-derived MSCs into patients with septic shock up to a dose of 3×10^6 cells/kg appears safe [49]. In accordance with the CISS study, our single-center clinical trial (ChCTR-TRC-14005094) data also show that a single intravenous infusion of allogeneic MSCs up to a dose of 3×10^6 cells/kg was safe and well tolerated in patients with severe sepsis [50]. Although MSCs have been shown to have some safety in the treatment of sepsis, the evidence of effectiveness is not sufficient.

17.3.2.1 Anti-inflammatory Effects

Mesenchymal stem cells protect the host via inhibiting the immune response to sepsis. The immune system is activated by bacterial infection and appears as an initial proinflammatory cascade that peaks within days of the inciting infection. While the institution of aggressive supportive therapies now permits the majority of patients with sepsis to survive this initial proinflammatory phase, organ damage

Table 17.1 Preclinical studies of mesenchymal stem cells for treatment of sepsis

Author [ref.]	Animal model	MSC source	MSC dose regimen	Trial length	Outcome
Gupta et al. [40]	Mouse *E. coli*-induced ALI	Mouse BMSC	7.5×10^5 intrapulmonary 4 h post *E. coli* challenge	48 h for survival	↑Survival, IL-10 ↓Pulmonary edema, alveolar epithelial permeability, TNF-α and MIP-2
Nemeth et al. [19]	Mouse CLP-induced sepsis	Mouse BMSC	1×10^6 IV 24 h prior or 1 h post-CLP	4 days for survival 6 h for ex vivo macrophage study 24 h for cytokines and vascular leakage	↑Survival, liver/kidney function, IL-10 ↓Proinflammatory cytokines, pancreatic inflammation, apoptosis/necrosis in the spleen, vascular leakage in the liver/kidney, neutrophil transmigration/activity
Gonzalez-Rey et al. [29]	Mouse CLP-induced sepsis	Human ADSC Mouse ADSC	1×10^6 IP 4 h post-CLP	10 days for survival 24 h for bacterial burden 18 h for others	↑Survival ↓Proinflammatory cytokines, neutrophil transmigration/activity, bacterial burden in the liver/spleen
Gonzalez-Rey et al. [29]	Mouse LPS-induced sepsis	Human ADSC	1×10^6 30 min post-LPS-injection	4 days for survival 6 h for others	↑Survival, anti-inflammatory IL-10 ↓Proinflammatory cytokines, neutrophil transmigration/activity
Mei et al. [34]	Mouse CLP-induced sepsis	Mouse BMSC +/− antibiotics	2.5×10^5 IV 6 h post-CLP	28 h for tissues analysis 7 days for survival 22 h for bacterial clearance	↑Survival, bacterial clearance, organ function ↓Proinflammatory cytokines, lung inflammation/injury

Table 17.1 (continued)

Author [ref.]	Animal model	MSC source	MSC dose regimen	Trial length	Outcome
Weil et al. [8]	Rat LPS-induced sepsis	Mouse BMSC	2×10^6 IP 1 h post-LPS-injection	6 h for serum analysis	↓Proinflammatory cytokines ↑Anti-inflammatory IL-10, cardiac function
Yagi et al. [41]	Rat LPS-induced sepsis	Human MSC	0.5×10^6 IM 0 min post-LPS-injection	48 h for infiltration of inflammatory cells	↑Liver/kidney function, IL-10 ↓Lung injury, liver inflammation
Manukyan et al. [42]	Rat LPS-induced sepsis	Mouse BMSC	2×10^6 IP 1 h post-LPS-injection	6 h for serum analysis	↑♀ > ♂ MSC, cardiac function, bcl-xL/bax ratio ↓♀ = ♂ MSC, proinflammatory cytokines
Weil et al. [18]	Rat LPS-induced sepsis	Mouse BMSC	2×10^6 IV 1 h post-LPS-injection	6 h for cardiac function and serum analysis	↓Proinflammatory cytokines ↑Anti-inflammatory IL-10, cardiac function
Kim et al. [43]	Mouse *E. coli*-induced ALI	Human UC-MSC	1×10^5 intrapulmonary 3 h post *E. coli* challenge	7 days for survival 1, 3, 7 days for others	↑Survival, bacterial clearance, organ function ↓TNF-α, IL-6, IL-1α, IL-1β, IL-6, MIP-1α, MIP-1β, MIP-2, RANTES, MPO
Chang et al. [44]	Rat CLP-induced sepsis	Rat ADSC (auto) Rat ADSC apoptotic (auto)	1.2×10^6 IV 0.5, 6, and 18 h after CLP	72 h for survival and others 96 h for detect ADMSCs	↑Survival, Tregs (healthy only) ↓Tregs (apoptotic only), TNF-α (apoptotic only)
Li et al. [45]	Rat LPS-induced ALI	Human UC-MSC	5×10^5 IV 1 h post-LPS	6, 24, and 48 h for serum and histology analysis 48 h for survival	↑Survival ↓IL-1β, TNF-α, Il-6, MPO No change: IL-10

(continued)

Table 17.1 (continued)

Author [ref.]	Animal model	MSC source	MSC dose regimen	Trial length	Outcome
Zhao et al. [46]	Rat LPS + chest impact-induced ALI	Rat BMSC	2.5×10^6 IV 2 h post-LPS	24, 48 h for survival	↑Survival, IL-10 ↓TNF-α, IL-6 No change: IL-1β
Sepulveda et al. [47]	Mouse LPS-induced sepsis	Human BMSC	1×10^6 IV 0.5 h post-LPS	6 days for survival 6 h for others	↑Survival ↑TNF-α, IL-6, IL-10

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BMSC bone marrow-derived mesenchymal stem cell, *UC-MSC* umbilical cord-derived mesenchymal stem cell, *ADSC* adipose-derived stem cell (modified from reference [3, 48], with permission from Elsevier)

resulting from this phase remains a remarkable cause of morbidity. Mesenchymal stem cells ameliorate this potential injury through an overall reduction in both local and systemic inflammation via a balanced increase in anti-inflammatory cytokine production and a decrease in proinflammatory cytokine production [8, 29, 48]. MSC-mediated upregulation of proinflammatory cytokines IL-1, IL-6, and TNF-α contributes to the diminished inflammatory milieu [8, 37]. Important players in the anti-inflammatory cytokine profile of MSCs include TNF-α stimulated gene/protein 6 (TSG-6), IL-10, IL-13, and transforming growth factor (TGF)-β [6, 36]. The reduced inflammatory state is an even more important consideration for the cytokine dysregulation observed in sepsis.

In sepsis, the lungs are especially sensitive to injury, and intra-alveolar neutrophil-mediated inflammation plays a marked underlying mechanism. In the gut, a septic mice experiment showed that human adipose-derived MSCs significantly reduced the septic inflammatory response and mortality through decreasing proinflammatory cytokine expression as well as increasing anti-inflammatory IL-10 [29]. A number of acute lung injury studies have confirmed that mesenchymal stem cells reduce lung inflammation through inhibiting the transmigration of neutrophils into alveoli [9, 51–53] and have significantly improved survival [40]. Several studies have shown that prostaglandin E2 (PGE_2) is an essential soluble factor secreted by MSCs to achieve their anti-inflammatory effects as well [19, 37, 54–57]. Furthermore, these cytokine effects could also be achieved from MSC-conditioned medium alone, thereby emphasizing their definitive paracrine features. Another study further emphasized the importance of PGE_2 secreted by MSCs in stimulating IL-10 production by resident macrophages in order to alleviate the excessive inflammatory response observed in sepsis [19]. This upregulation of macrophage IL-10 synthesis was responsible for an improvement in renal and liver function and increased survival rates.

MSCs may also inhibit proinflammatory mediator expression from innate immune cells in part via a negative feedback loop. This inhibition of macrophage proinflammatory cytokine production essentially short-circuits the early intraperitoneal inflammatory response in sepsis, and MSC-secreted TSG-6 represents yet another anti-inflammatory factor. A recent study confirmed that the interaction of MSC-secreted TSG-6 with the CD44 receptor of resident macrophages effectively downregulated nuclear factor (NF)-κB signaling to reduce expression of TNF-α [36]. These studies have offered greater insight into the complexity of MSC anti-inflammatory mechanisms in multiple organ injuries and may provide novel strategies in the management of sepsis.

17.3.2.2 Anti-apoptotic Effects

An anti-inflammatory response may be beneficial in early sepsis; however, sepsis also induces an inappropriate immunosuppression, which serves unclear purpose as the syndrome progresses. Similarly, a great deal of apoptotic events benefit to an organism in response to damage; however, apoptosis seen in sepsis also lacks definitive benefit. This cell death is independent of direct infectious agent contact but is through cytokines such as TNF which activate caspase systems. Septic environment results in a widespread apoptosis of cells with high turnover rates such as gastrointestinal cells and lymphocytes. This system-wide apoptosis leads to the profound lymphopenia which associates with poor sepsis outcomes and helps establish sepsis secondary to immunosuppressive phase, which is often fatal. Thus, many studies are underway to prevent apoptosis in progressing sepsis [58].

MSCs have the capacity to assuage this apoptosis. In several myocardial infarction/reperfusion studies, it is confirmed that mesenchymal stem cells are capable of improving the survival of threatened cells along the border of the myocardial infarct zone [6, 59–61]. Stem cells possess anti-apoptotic mechanisms such as increasing antioxidant activity, downregulating mitochondrial death pathways, upregulating DNA repair, and altering anti- and pro-apoptotic protein expression [62–64]. These mechanisms would be even more important in sepsis, where oxidative stress, mitochondrial damage, and apoptosis have clearly been implicated in pathology [65–67]. Another study showed that MSCs significantly decrease the ratio of pro-apoptotic bax to anti-apoptotic bcl-xL in endotoxemic cardiac tissue, which correlated with reduced cardiac dysfunction [42]. Recently, Yagi et al. revealed that MSCs significantly reduced the number of apoptotic cells found in the lungs and kidneys of endotoxemic rats [41]. While prior MSC studies have confirmed sexual dimorphism between female and male donor MSCs [68, 69], this study is the first to demonstrate the greater efficacy of female MSCs in an endotoxemia model. Similarly, Mei et al. demonstrated the capacity of MSCs to prevent apoptotic cell death in the lung and kidneys of CLP mice [34]. As respiratory failure and renal failure represent key limiting factors in predicting post-sepsis survival, employing MSC-based therapy to alleviate injury to these organs is an attractive possibility. While MSC-mediated alterations in the bcl-xL/bax ratio have been confirmed in infarction/reperfusion and

endotoxemia models, ongoing study will be needed to evaluate whether MSCs exert anti-apoptotic effects by regulation of mitochondrial death pathways and caspases in sepsis as has been shown in the ischemia/reperfusion model [70, 71].

17.3.2.3 Neoangiogenic Effects

The neoangiogenic properties of MSCs are still being elucidated, but several studies have revealed angiogenic cytokines secreted by MSC [38, 72, 73]. MSC-secreted paracrine factors like basic fibroblast growth factor (FGF2), VEGF, angiopoietin (Ang)-1, and hepatocyte growth factor (HGF) promote neovascularization of injured organs [10, 68, 74]. This may be particularly important in sepsis, where coagulation dysfunction and microvascular congestion produce multi-organ ischemia. It has been demonstrated that LPS, TNF-α, or hypoxia exposure increase the quantity of HGF, FGF2, and VEGF produced by MSCs, which enhance their neoangiogenic potential upon injured organs [75, 76]. Tang et al. demonstrated that MSCs assuaged the effects of myocardial infarction in a rat model by increasing vascular regeneration through secretion of SDF-1, FGF-1, and VEGF [77]. Another study also revealed that the neoangiogenic activity of MSCs is induced by hypoxia and is contingent upon a balanced ratio of MSC-secreted pigment epithelial-derived factor (PEDF) to VEGF [78]. Several other studies have revealed that MSCs improve tissue neoangiogenesis by secreting soluble factors to promote endothelial cell sprout formation [79, 80]. MSCs increase their secretion of angiogenic growth factors in infarction/reperfusion injury and when exposed to inflammatory mediators or LPS in vitro studies. Consequently, it is likely that MSCs exert a similar paracrine mechanism promoting their neoangiogenic potential in sepsis.

17.3.2.4 Activation of Resident Stem Cells

The panoply of growth factors secreted by MSCs is critical for vascularity potential but may also mobilize the resident stem cell populations in the adult heart, liver, kidney, and lung [81]. VEGF is a key mobilizer of cardiac stem cells, which improved cardiac function following acute regional infarction [82]. As these organs are affected deleteriously in the patients with sepsis, the possibility of MSC-secreted HGF, VEGF, and insulin-like growth factor (IGF)-1 to stimulate resident stem cell proliferation provides another potential mechanism by which MSCs may improve the morbidity of organ dysfunction [6, 10]. More recently studies revealed that MSC-secreted VEGF is a critical paracrine factor to mediate cardiac regeneration [83], and it is possible that this is achieved in part through a VEGF-dependent mobilization of cardiac stem cells [84]. Mazhari et al. demonstrated that MSCs could mobilize an endogenous population of cardiac stem cells through cell-to-cell interactions and complex paracrine to improve ejection fraction after myocardial infarction [39]. As end-organ hypoxia plays one of the key derangements underlying the pathophysiology of sepsis, these infarction studies offer insight into how MSCs may promote the mobilization of resident stem cells in a variety of injured organs. Further research will be needed to fully evaluate to what extent MSCs affect the proliferation and mobilization of resident stem cell populations in other organ systems in sepsis.

17.3.2.5 Immunomodulatory Effects

A large number of studies reported that MSC exerts a role in immune regulation. The study found that it can play an immunomodulatory effect on immune cells such as antigen-presenting cells, B cells, NK cells, T cells, regulatory T cells, macrophages, and other immune cells. IL-4, IL-10, TGF-β, hepatocyte growth factor (HGF) and nitric oxide (NO), PGE2, and indoleamine 2, 3-dioxygenase (IDO) are involved in the immunoregulation of MSC.

A variety of immune cells and MSCs, in addition to the secretion of a large number of soluble cytokines to regulate the inflammatory response, can also inhibit a variety of immune cell subsets of specific and non-specific immunity to regulate the body's immune function, showing multidirectional immunity regulatory activity [85]. These immune cells include different effector cells such as CD8+ cytotoxic T cells (CTL), Tregs [86, 87], natural killer cells (NK) [54, 88], and special types of T cells (NKT cells and γδ cells [89]) and potential antigen-presenting cells (APC) (such as DCs [90] and B cells [91], macrophages [92]).The immunomodulatory mechanism of MSC is summarized in Fig. 17.1.

Effect of MSC on Neutrophils

Neutrophils as one of the major effector cells of innate immunity are polymorphonuclear neutrophils (PMNs) that aggravate the tissue inflammation. Studies have shown that MSCs can inhibit the PMN respiratory burst (oxygen burst) through IL-6-dependent pathway and their migration and inflammatory response. At the same time, MSCs can inhibit PMN apoptosis and prolong their life. In addition, it has been

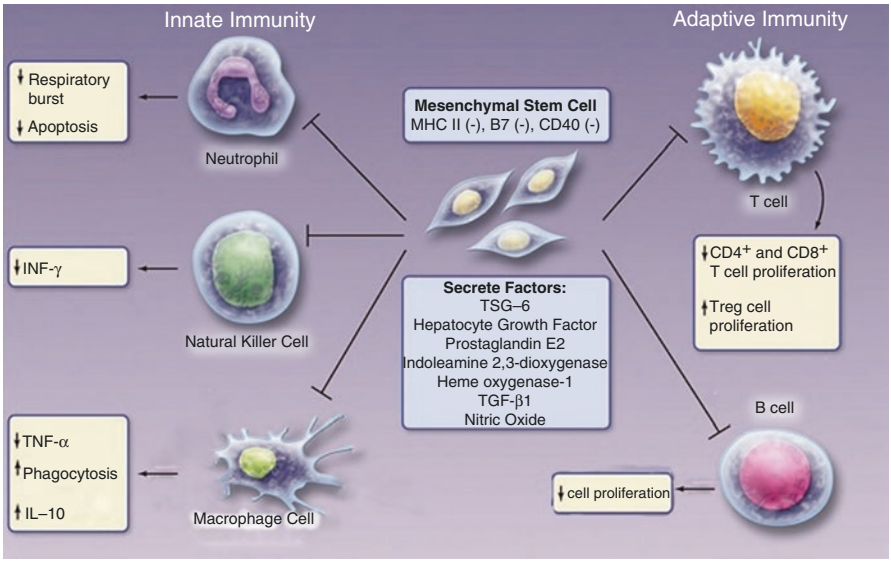

Fig. 17.1 The role of MSC in immunomodulatory (modified from reference [93], with permission from Wolters Kluwer Health). MSCs are immune exemption cells that can suppress the innate immunity (neutrophils, macrophages, and NK cells) and acquired immunity (T cells and B cells)

found that MSCs can induce T helper cells 2 (Th2) (CD4+ CD45RO+ T cells) to produce IL-17 which enhance the phagocytosis of neutrophils, so that neutrophils play a role in the elimination of pathogens rather than inflammation effect [84].

Effect of MSC on Antigen-Presenting Cells

Dendritic cells (DCs) are the most important antigen-presenting cells that activate, maintain, and regulate immune responses by promoting the activation of antigen-specific T cells and activating the innate immune system. At present, the effect of MSC on DC is mainly focused on the differentiation and maturation of DCs from monocytes. Nauta et al. have shown that MSC can downregulate the expression of costimulatory molecules CD40, CD80, CD83, and CD86 of DC and prevent CD4 monocytes and CD34 progenitor cells differentiated to DC [94]. Later, Spaggiari et al. demonstrated that MSC inhibits immune responses by inhibiting the maturation of DCs via secreting PGE2 which inhibit monocytes differentiated to immature DCs [90]. Barbara et al. found that compared with adipose-derived MSC and renal tubular epithelial cells, amniotic fluid-derived MSCs increased IL-10 and down-regulated IL-12 and P70 by producing PGE_2, to completely eliminate mononuclear cells to DC differentiation resulting in the reduction of molecular surface of CD80, CD86, and HLA-DR [93]. However, it has also been reported that umbilical cord-derived MSCs can not only inhibit the differentiation of monocytes to immature DC cells but also promote the maturation of DC cells [95]. The different results may be related to the different sources of MSCs, suggesting that different sources of MSCs have different immunomodulatory effects.

DCs play an important role in the initiation, maintenance, and regulation of immune responses by promoting antigen-specific T cell activation and activating non-specific immune cells. In recent years, the role of MSCs in DCs has also attracted attention. MSCs can downregulate the expression of CD40, CD80, CD83, and CD86 in DCs and inhibit the differentiation of CD14+ monocytes and CD34+ precursor cells into DCs under in vitro conditions [90]. There is no sufficient evidence that MSCs are through the mechanisms that inhibit the maturation of DCs, but studies suggest that PGE_2 may affect early maturation of DCs by altering the expression of surface molecules CD80, D60, and CD83 and producing IL-12. Under normal circumstances, mature DCs highly express MHC-II and the antigen peptide-MHC class II molecule complex can be presented to the initial T cells. However, under the action of MSCs, DCs secrete IL-12 and IFN-α, IL-1β and IL-10 increased, and MHC-II antigen expression levels decreased [87, 90]. MSCs inhibiting the expression of MHC-II in DC will reduce its antigen presentation function.

In the sepsis mouse model, MSCs were affected by the proliferation, activation, and secretion of various immune cells. The regulation of the immune system of MSCs was not a single action on the inflammatory response which make MSCs a hopeful immune modulators.

Immunosuppressive Effects of MSC on T Cells

T cells are the other major effector cells of adaptive immunity. MSCs can inhibit the proliferation of T cells while stimulated by allogeneic antigens, mitogen, and CD3

and CD28 antibodies. Studies have shown that this inhibitory effect is mainly mediated by soluble factors such as TGF-β, HGF, and IDO, in which IDO is thought to play a major role in the inhibition of T cell proliferation by MSCs. IFN-γ stimulates MSCs to secrete IDO, which degrades tryptophan leading to kynurenine production, thereby inhibiting cell proliferation and even mediating T cell apoptosis. Immunosuppressive function regulatory T cells (Treg) are different from T helper cells 1 (Th1) and Th2, with a regulatory function of mature T cell subsets. MSCs can also stimulate the proliferation of Treg cells by releasing human leukocyte antigen-G5 (HLA-G5) and TGF-β1 [96, 97].

In vitro experiments showed that MSCs were able to inhibit T cell proliferation induced by mitogen, allogeneic antigen, LPS, and so on. One important feature of MSCs to inhibit T cell immune responses is that they are not affected by MHC, and both MSCs from donors, receptors, and third parties can induce this effect [86] in a dose-dependent manner [92, 98, 99]. The studies found that MSCs downregulated the expression of cyclin D2, upregulated the expression of p27Kip1, and blocked T cells in the G0/G1 cell cycle, and exogenous cytokine D2 could reverse the inhibitory effect of MSCs on T cells, suggesting that MSCs play an immunosuppressive role by inhibiting the proliferation of T cells [100].

MSCs can affect the function of T cells by regulating the levels of various cytokines. IFN-γ, TNF-α, anti-inflammatory factors IL-4, IDO, and TGF-β [85, 101, 102], these immunosuppressive factors can further inhibit the activation and function of T cells. Although MSCs strongly inhibit the secretion of IFN-γ [100], IFN-γ has an irreplaceable effect in initiating the immunosuppression of MSCs. BMSCs that knock out IFN-γ R gene or IFN-γ can't inhibit the proliferation of T cells [101].

Immunosuppressive Effects of MSC on B Cells

B cells are the main effector cells of humoral immunity, while the proliferation, apoptosis, immunoglobulin (Ig) secretion, and chemotaxis of B cells are affected when co-cultured with MSCs. When allogeneic MSCs co-cultured with B cells, the stimulating response of B cells to mitogen can be completely inhibited, and the blocking antibody of the programmed cell death molecules (PD-1, PD-L1, and PD-L2) could partly recover B cell proliferation stimulated by antigen [103]. MSCs can regulate the secretion of Ig by B cells both in vivo and in vitro. Co-culture at 1:10 (MSCs: B cells) resulted in an increase in IgG secretion, but the secretion of IgG was significantly decreased when the two cells did not direct contact. In addition, MSCs can inhibit the secretion of IgM, IgG, and IgA at a high ratio (1:1, MSCs: B cells) and significantly reduce the expression of CXCR4, CXCR5, and CXCR7, thereby inhibiting the chemotactic activity of B cells and weakening humoral immune response.

In rat experiments, MSCs inhibited B cell proliferation after CD40L antibody, IL-4 [37], or mitotic [104] activation. Krampera et al. [105] have reported that human BMSCs can inhibit the proliferation of B cells activated by anti-immunoglobulin antibodies, soluble CD40 ligands, and cytokines. Although the mechanisms of these phenomena have not been fully elucidated, this may be related to IFN-γ-induced IDO produced by BMSCs. Prigione et al. [89] have also confirmed

that the soluble factor produced by BMSCs can inhibit B cell proliferation. MSCs can block the proliferation of activated B cells induced by IFN-γ and inhibit the production of antibodies. Its inhibitory effect on the secretion of antibodies is dose-dependent and is associated with the state of B cell activation [105, 106]. In addition, MSCs affect the migration of B cells in immune responses by inhibiting the secretion of chemokines [91].

Immunosuppressive Effects of MSC on NK Cells

Presently, it is confused for the immunosuppressive effects of MSC on NK cells. Wang et al. [107] showed that small doses of MSC could promote the proliferation of NK cells and enhance the antitumor capacity of NK cells, whereas large doses of MSC were diametrically opposed to suppressing the proliferation of NK cells and weakening NK cells' antitumor capacity. Other studies [16, 95] demonstrated that MSCs can inhibit the proliferation of NK cells and attenuate the cytotoxicity of NK cells in vitro, thereby weakening NK cells' antitumor capacity. According to Thomas et al. [54], MSCs co-cultured with NK cells enhanced IL-12/IL-18-induced NK cells release of IFN-γ, thereby enhancing the ability to resist infection during injury. MSC on NK cell immunosuppressive effect has not yet reached a consensus; MSC regulation of NK cell-specific mechanism remains to be further studied.

MSCs can inhibit the proliferation of NK cells, reduce the secretion of IFN-γ, and affect the expression of its surface cell molecules [37]. MSCs inhibit IL-2-activated NK cell proliferation, which may be primarily related to the direct effect of soluble immunosuppressive factors such as TGF-β, HLA-G, PGE_2 and cells [85, 108]. Effector functions of natural killer cell (such as anti-virus cell cytotoxic effect) can also be inhibited by MSCs, and accompanied by reduced IFN-γ secretion, which is mainly secreted with MSCs PGE_2, IDO weakened NK cell activation receptor about [54]. Although studies have reported that MSCs can't inhibit the dissolution of isolated NK cells [98], Krampera et al. [105] found that the cytotoxicity of NK cells was attenuated by MSCs and MSCs were inhibited by HLA-I-positive NK cells. The effect was more pronounced than HLA-I-negative NK cells [108].

Mixed BMSCs and lymphocytes with 1: 1 ration can inhibit cytotoxic T cells (CTL) and NK cells cytotoxic function [86], indicating that BMSCs can downregulate CD8+ T cells and NK cells at very high concentrations and this high concentration of BMSCs can't be used in vivo. Studies on the interaction between NK cells and MSCs [88] have also found that not only MSCs inhibit the proliferation of NK cells but also activate NK cells to kill MSCs.

Effect of MSC on Treg Cells

Treg cells are now recognized as immunosuppressive cell populations. It has been reported that MSC co-cultured with CD4+ T cells can induce the proliferation of CD4+ CD25+ FOXP3+ Treg cells and CD8+ Treg cells [108], and this effect was depended on TFG-β and PGE_2 [86]. Casiraghi et al. [85] demonstrated that MSC play an immunomodulatory role by inducing the production of Treg cells and

inhibiting the occurrence of transplant rejection. Chang et al. [109] also demonstrated that MSCs play an immunosuppressive effect through increasing the number of Treg cells to inhibit T cell proliferation, and this inhibitory effect was reinforced in the presence of IFN-γ.

MSCs can induce the expression of FOXP3 on CD4+ CD25+ Tregs [86, 102, 110] and CD8+ Tregs [86], maintain the expression of Fox3 on Tregs, and downregulate the expression of CD127 on Tregs [111]. Tregs are immunoregulatory T cell populations, especially CD4+ CD25+ Tregs, which have immunological incompetence and immunosuppressive function and play an important role in inducing peripheral immune tolerance. Studies [106, 112] found that factors affecting MSC-induced Tregs production include the interaction of MSCs with CD4+ T, cytokines PGE_2, TGF-β, and osteoproteins 2.

In addition, there are studies [113–115] that MSCs constitutively express human albumin antigen G (HLA-G) and costimulatory molecules B7-H4, which inhibit T cell activation, proliferation, and/or T cell mediated of the cytotoxic effect. The addition of HLA-G or B7-H4 antibody could significantly reverse the inhibitory effect of MSCs on T cell proliferation, suggesting that these two molecules play an important role in the immunosuppression of MSCs.

Effect of MSC on Macrophages

Recent studies have shown that one of the important functions of MSCs is to act as a "guardian" [116] against excessive inflammatory response. Macrophages are the main effector cells of innate immune responses, and MSCs can regulate their functional status in a variety of ways. Firstly, MSCs can strengthen macrophage phagocytosis. MSCs interact with macrophages to increase the expression of phagocytic function molecules in macrophages, such as scavenger receptors, which enhance the phagocytic function of macrophages and reduce the number of invasive pathogens. On the other hand, the reduction of the pathogen composition to stimulate the immune cells reduced the local inflammatory injury and systemic inflammatory response. Secondly, in the inflammatory microenvironment, macrophage-derived tumor necrosis factor-α (TNF-α) and other proinflammatory factors can stimulate MSC secretion of multidrug-resistant anti-inflammatory protein tumor necrosis factor-inducible protein 6 protein (TSG-6) [117]. TSG-6 downregulates the activity of NF-κB signaling pathway by interacting with CD44 receptors on macrophages. By this negative feedback loop, reduction of TNF-α and other proinflammatory cytokines weakened the proinflammatory cascade by macrophages.

Furthermore, the regulation of macrophage immune function by MSCs can be mediated by the secretion of PGE_2. The bacterial lipopolysaccharide (LPS) binds to the surface of the MSCs with Toll-like receptor 4 (TLR4) when a large number of gram-negative (G-) bacteria invade. At the same time, these cells are stimulated by macrophage-secreted TNF-α (second signal), which binds to MSC surface receptor tumor necrosis factor receptor 1, initiated signaling pathways involving myeloid differentiation factor 88 (Myd88) and NF-κB, upregulated cyclooxygenase-2 (COX-2), and then catalyzed PGE_2 synthesis increase. Thus, PGE_2 derived from MSCs binds to E2 and E4 receptors on the surface of

macrophages through cell-to-cell contact-dependent pathways, promoting macrophage phenotypic transformation from proinflammatory phenotype to anti-inflammatory phenotype, resulting in a large number of anti-inflammatory factors IL-10 productions, which is called macrophage reprogramming. Reprogrammed macrophages secreting IL-10 can significantly reduce neutrophil exudation and migrate to infected tissues, limiting neutrophil-mediated tissue damage.

Krasnodembskaya et al. [98] showed that the levels of proinflammatory/anti-inflammatory factors in serum of sepsis mice were not significantly altered after MSCs treatment, but the survival rate of mice was significantly higher than that of the control group. Not only through the regulation of anti-inflammatory/proinflammatory response to play a curative effect, MSC treatment also reduced the blood Pseudomonas aeruginosa colonies of CLP mice, and enhanced phagocytosis activity of monocytes. In addition, the level of C5a in the serum of the treatment group was significantly higher, which may be related to the upregulation of CD11b receptor expression on macrophages. These results suggest that the increased survival rate of MSCs in sepsis mice is due to the increased ability of MSCs to engulf bacteria. Nemeth et al. [19] also found that CLP mice treated with MSCs have l elevated level of mononuclear macrophages in the plasma. However, after treated with a liposomal containing disodium chloride quinate in vivo which mainly to eliminate mononuclear cells and macrophages, the beneficial effect of MSCs disappeared, suggesting that the therapeutic effect of MSCs to improve the survival rate of sepsis mice depend on the presence of monocytes/macrophages and the secretion of anti-inflammatory factor IL-10.

In conclusion, a large number of studies have confirmed that MSCs can regulate the proliferation, functional status, and phenotype transformation of different immune cells in vitro and in vivo. Although the immunosuppressive effects of MSCs remain to be fully elucidated and possibly represent a two-edged sword, many studies have confirmed the biological function of MSCs, indicating a good application prospects.

17.3.3 Key Cytokines Affect Therapeutic Effect of Exogenous MSC on Sepsis

Studies [117] show that MSCs can exert immunosuppressive effects through cell-dependent and non-cell-dependent mechanisms. Nemeth et al. [19] hold that MSCs can regulate the level of proinflammation/anti-inflammatory factors in the blood, thereby improving the local and systemic inflammatory response and improving the survival rate of sepsis mice. MSCs secrete various biological activating factors to alter the secretion phenotype of various immune cells, downregulate the inflammatory factors in microenvironment, and direct the immune response such as homing and migration of MSCs. MSCs can promote immune cells to secrete a variety of anti-inflammatory factors, including IL-10, PGE_2 and IDO in the treatment of sepsis [37, 118].

17.3.3.1 IL-10

Nemeth et al. [19] using mouse CLP sepsis animal model study found that sepsis mice model 1 h after injection of MSCs can significantly improve the mouse 24-, 48-, 72-, 96-h survival rate and increase plasma IL-10. However, the beneficial effects of MSCs disappeared if blocking IL-10, suggesting that IL-10 plays an important role in the mechanism of MSCs in the treatment of sepsis mice. However, MSCs still have therapeutic effects in the treatment of sepsis mice using IL-10 knockout MSCs, suggesting that IL-10 may play a role in sepsis rather than MSCs. Further studies [119] found that MSCs can promote monocyte/macrophage secretion of IL-10 in mice.

IL-10 can be secreted by Th2 cells, partially regulated T cells (Tregs), monocytes, and macrophages. IL-10 could not only inhibits Th1 cell responses and synthetic cytokines, macrophage antigen presentation and cytokine synthesis such as IL-2, TNF-α, IL-12, and IFN-γ, but also downregulates the expression of MHC-I [120, 121]. Studies have shown that IL-10 can inhibit neutrophil scrolling [122] and migrate through endothelial cells into tissues [123]. IL-10 can neutralize neutrophils in the circulatory system, which facilitates the removal of bacteria in the bloodstream and reduces the oxidative damage caused by neutrophils in the removal of bacteria in tissues [124].

17.3.3.2 PGE_2

To play an anti-inflammatory role in the inflammatory environment, inflammatory cytokines such as TNF-α and IFN-γ could induce MSCs secreting PGE_2. Besides, MSCs and concanavalin A co-culture can also secrete a large number of PGE_2 [125]. PGE_2 has an effect on many tissues and immune systems, in which T cells, dendritic cells (DCs), and macrophages are most affected by PGE_2. The recent study [126] reported that PGE_2 can inhibit the release of intracellular calcium associated with p59 protein tyrosine kinase activity which directly affects the proliferation and secretion of T lymphocytes (such as IL-12, TNF-α, etc.). PGE_2-stimulated EP2 and EP4 (PGE_2 receptor subtypes) that can promote the maturation of monocyte-derived DCs; EP2 and EP4 activation can be upregulated by yeast polysaccharide-induced IL-10 [127]. A further study from Hata et al. [128] has found that the co-exist of EP2 and EP4 determine the expression of IL-10. However, EP2 or EP4 deficiency will affect the production of IL-10, but the mechanism is not clear.

17.3.3.3 IDO

IDO also plays an important role in MSCs to improve the survival rate of sepsis model. In general, human MSCs do not produce IDO, but in vitro experiments have shown that MSCs express IDO increases under specific conditions [129]. IDO is the pyrrole epoxidation cleavage in the tryptophan molecule, and the rate-limiting enzyme metabolized by the tryptophan pathway can inhibit the proliferation of T cells and NK cells by degrading tryptophan and promote immune tolerance [105]. Studies have shown that IDO plays an important role in the maturation of Tregs [130] and DCs [131]. T cells are extremely sensitive to the degradation of tryptophan, in the absence of tryptophan under the conditions of T cell stagnation in the G1 medium. The possible mechanism by which MSCs generate IDO1 is that LPS

stimulates MSCs TLR3 and TLR4 activation and thereby the activation of PKR, autocrine IFN-β signaling, and the activation of STAT1/IRF-1 [118]. Studies [132] show that tryptophan cannot restore the proliferation of T cells, and tryptophan metabolite kynurenine blockers can reverse the immune response of mixed lymphocytes in MSCs [133]. This suggests that IDO-induced immunosuppression may rely on tryptophan degradation downstream metabolites [134].

17.4 Conclusion

Sepsis remains a most common complication of serious burning and trauma, shock, infection, and surgery and carries a high degree of morbidity and mortality with associated fiscal burden to the world healthcare economy. The further development of sepsis can lead to secondary septic shock, multiple organ dysfunction syndrome (MODS), which is one of the most important causes of death in clinical critically ill patients. With the development of stem cell research, the evidences show that all kinds of endogenous stem cell appear as dysfunctional state during or after sepsis, which plays an important role in the development and prognosis of sepsis. Thus, how to protect from endogenous stem cell injury may be a novel promising strategy for sepsis treatment.

Actually, while there are no multicenter random clinical trials reporting the role of MSCs in sepsis to date, an expanding field of experimental research using animal models suggests that MSCs may exert a unique ability to modulate various aspects of the complex pathophysiology in human sepsis. Animal experimental data show that MSCs possess the ability to home injured tissue, inhibit apoptosis in injured tissue, reduce the deleterious activities of neutrophils in injured tissue, reduce the inflammatory response locally and systemically, activate resident stem cell populations, stimulate neoangiogenesis, favor the formation of regulatory lymphocytes, and enhance bacterial clearance, among others. In addition, benefits achieved by ex vivo preconditioning and genetic modifications of MSCs have yet to be explored in more sepsis studies but have displayed great potential in other disease models. Investigating into the timing of MSC administration and further revealing the mechanisms of MSC immunoregulation in sepsis are keys to future successes. The potential to condition or activate specific MSC immunoregulatory profiles to guide therapies in sepsis and across many disease fields remains promising. As recent animal models of sepsis have revealed the ability of MSCs to significantly inhibit the excessive inflammatory response of sepsis as well as the multi-organ dysfunction observed in the later phase of sepsis, MSC clinic administration is considered to be a promising cell-based therapy, which continues to merit further investigation. Indeed, the interaction of MSC with the immune system at multiple levels may provide a dynamic novel treatment for the future septic patient. Although MSCs have been shown to have some safety in the treatment of sepsis, the evidence of effectiveness is not sufficient, and more multicenter random clinical trials need to be developed to evaluate the role of MSCs and establish the standardized clinical guideline in septic patient in the future.

References

1. Angus DC, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29(7):1303–10.
2. Levy MM, et al. The surviving sepsis campaign: results of an international guideline-based performance improvement program targeting severe sepsis. Intensive Care Med. 2010;36(2):222–31.
3. Wannemuehler TJ, et al. Advances in mesenchymal stem cell research in sepsis. J Surg Res. 2012;173(1):113–26.
4. Rocheteau P, et al. Sepsis induces long-term metabolic and mitochondrial muscle stem cell dysfunction amenable by mesenchymal stem cell therapy. Nat Commun. 2015;6:10145.
5. Trounson A, et al. Clinical trials for stem cell therapies. BMC Med. 2011;9:52.
6. Crisostomo PR, et al. Surgically relevant aspects of stem cell paracrine effects. Surgery. 2008;143(5):577–81.
7. Ceradini DJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med. 2004;10(8):858–64.
8. Weil BR, et al. Mesenchymal stem cells attenuate myocardial functional depression and reduce systemic and myocardial inflammation during endotoxemia. Surgery. 2010;148(2):444–52.
9. Xu J, et al. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. Am J Physiol Lung Cell Mol Physiol. 2007;293(1):L131–41.
10. Wang M, et al. Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. Am J Physiol Regul Integr Comp Physiol. 2006;291(4):R880–4.
11. Fletcher SN, et al. Persistent neuromuscular and neurophysiologic abnormalities in long-term survivors of prolonged critical illness. Crit Care Med. 2003;31(4):1012–6.
12. Zhang H, et al. Sepsis induces hematopoietic stem cell exhaustion and myelosuppression through distinct contributions of TRIF and MYD88. Stem Cell Reports. 2016;6(6):940–56.
13. Bosmann M, Ward PA. The inflammatory response in sepsis. Trends Immunol. 2013;34(3):129–36.
14. Rodriguez S, et al. Dysfunctional expansion of hematopoietic stem cells and block of myeloid differentiation in lethal sepsis. Blood. 2009;114(19):4064–76.
15. Esplin BL, et al. Chronic exposure to a TLR ligand injures hematopoietic stem cells. J Immunol. 2011;186(9):5367–75.
16. De Miguel MP, et al. Immunosuppressive properties of mesenchymal stem cells: advances and applications. Curr Mol Med. 2012;12(5):574–91.
17. Shi Y, et al. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. Cell Res. 2010;20(5):510–8.
18. Weil BR, et al. Intravenous infusion of mesenchymal stem cells is associated with improved myocardial function during endotoxemia. Shock. 2011;36(3):235–41.
19. Nemeth K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15(1):42–9.
20. Ponte AL, et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells. 2007;25(7):1737–45.
21. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. Exp Biol Med (Maywood). 2001;226(6):507–20.
22. Segers VF, et al. Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. Am J Physiol Heart Circ Physiol. 2006;290(4):H1370–7.
23. Brooke G, et al. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. Stem Cells Dev. 2008;17(5):929–40.
24. Ringe J, et al. Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. J Cell Biochem. 2007;101(1):135–46.

25. Tondreau T, et al. In vitro study of matrix metalloproteinase/tissue inhibitor of metalloproteinase production by mesenchymal stromal cells in response to inflammatory cytokines: the role of their migration in injured tissues. Cytotherapy. 2009;11(5):559–69.
26. De Becker A, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. Haematologica. 2007;92(4):440–9.
27. Ries C, et al. MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood. 2007;109(9):4055–63.
28. Von Luttichau I, et al. Human adult CD34- progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. Stem Cells Dev. 2005;14(3):329–36.
29. Gonzalez-Rey E, et al. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut. 2009;58(7):929–39.
30. Zhang D, et al. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2008;44(2):281–92.
31. Hu X, et al. Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. Am J Physiol Cell Physiol. 2011;301(2):C362–72.
32. Li D, et al. Bone marrow mesenchymal stem cells suppress acute lung injury induced by lipopolysaccharide through inhibiting the TLR2, 4/NF-kappaB pathway in rats with multiple trauma. Shock. 2016;45(6):641–6.
33. Luo Y, et al. Pretreating mesenchymal stem cells with interleukin-1beta and transforming growth factor-beta synergistically increases vascular endothelial growth factor production and improves mesenchymal stem cell-mediated myocardial protection after acute ischemia. Surgery. 2012;151(3):353–63.
34. Mei SH, et al. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. Am J Respir Crit Care Med. 2010;182(8):1047–57.
35. Kim JS, et al. A novel therapeutic approach using mesenchymal stem cells to protect against mycobacterium abscessus. Stem Cells. 2016;34(7):1957–70.
36. Choi H, et al. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kappaB signaling in resident macrophages. Blood. 2011;118(2):330–8.
37. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815–22.
38. Kinnaird T, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94(5):678–85.
39. Mazhari R, Hare JM. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. Nat Clin Pract Cardiovasc Med. 2007;4(Suppl 1):S21–6.
40. Gupta N, et al. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol. 2007;179(3):1855–63.
41. Yagi H, et al. Bone marrow mesenchymal stromal cells attenuate organ injury induced by LPS and burn. Cell Transplant. 2010;19(6):823–30.
42. Manukyan MC, et al. Female stem cells are superior to males in preserving myocardial function following endotoxemia. Am J Physiol Regul Integr Comp Physiol. 2011;300(6):R1506–14.
43. Kim ES, et al. Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells attenuates *Escherichia coli*-induced acute lung injury in mice. Respir Res. 2011;12:108.
44. Chang CL, et al. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. J Transl Med. 2012;10:244.
45. Li J, et al. Human umbilical cord mesenchymal stem cells reduce systemic inflammation and attenuate LPS-induced acute lung injury in rats. J Inflamm (Lond). 2012;9(1):33.

46. Zhao Y, et al. Therapeutic effects of bone marrow-derived mesenchymal stem cells on pulmonary impact injury complicated with endotoxemia in rats. Int Immunopharmacol. 2013;15(2):246–53.
47. Sepulveda JC, et al. Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model. Stem Cells. 2014;32(7):1865–77.
48. Tanaka F, et al. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. Life Sci. 2008;83(23–24):771–9.
49. McIntyre LA, et al. Cellular immunotherapy for septic shock. A phase I clinical trial. Am J Respir Crit Care Med. 2018;197(3):337–47.
50. He X, et al. Umbilical cord-derived mesenchymal stem (stromal) cells for treatment of severe sepsis: a phase 1 clinical trial. Transl Res. 2018. https://doi.org/10.1016/j.trsl.2018.04.006.
51. Ortiz LA, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A. 2007;104(26):11002–7.
52. Lee JW, et al. Allogeneic human mesenchymal stem cells for treatment of *E. coli* endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci U S A. 2009;106(38):16357–62.
53. Mei SH, et al. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. PLoS Med. 2007;4(9):e269.
54. Spaggiari GM, et al. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood. 2008;111(3):1327–33.
55. English K, et al. Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25 (High) forkhead box P3+ regulatory T cells. Clin Exp Immunol. 2009;156(1):149–60.
56. Spaggiari GM, et al. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. Blood. 2009;113(26):6576–83.
57. Chen K, et al. Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE2-dependent mechanism. Clin Immunol. 2010;135(3):448–58.
58. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol. 2006;6(11):813–22.
59. Xu M, et al. In vitro and in vivo effects of bone marrow stem cells on cardiac structure and function. J Mol Cell Cardiol. 2007;42(2):441–8.
60. Hu X, et al. Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg. 2008;135(4):799–808.
61. Li W, et al. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells. 2007;25(8):2118–27.
62. Dernbach E, et al. Antioxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. Blood. 2004;104(12):3591–7.
63. Ramalho-Santos M, et al. "Stemness": transcriptional profiling of embryonic and adult stem cells. Science. 2002;298(5593):597–600.
64. Ivanova NB, et al. A stem cell molecular signature. Science. 2002;298(5593):601–4.
65. Pulido EJ, et al. Differential inducible nitric oxide synthase expression in systemic and pulmonary vessels after endotoxin. Am J Physiol Regul Integr Comp Physiol. 2000;278(5):R1232–9.
66. Hotchkiss RS, Tinsley KW, Karl IE. Role of apoptotic cell death in sepsis. Scand J Infect Dis. 2003;35(9):585–92.
67. Vanhorebeek I, et al. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet. 2005;365(9453):53–9.
68. Crisostomo PR, et al. Gender differences in injury induced mesenchymal stem cell apoptosis and VEGF, TNF, IL-6 expression: role of the 55 kDa TNF receptor (TNFR1). J Mol Cell Cardiol. 2007;42(1):142–9.

69. Crisostomo PR, et al. Sex dimorphisms in activated mesenchymal stem cell function. Shock. 2006;26(6):571–4.
70. Poynter JA, et al. Intracoronary mesenchymal stem cells promote postischemic myocardial functional recovery, decrease inflammation, and reduce apoptosis via a signal transducer and activator of transcription 3 mechanism. J Am Coll Surg. 2011;213(2):253–60.
71. Wang X, et al. Stem cells for myocardial repair with use of a transarterial catheter. Circulation. 2009;120(11 Suppl):S238–46.
72. Gnecchi M, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006;20(6):661–9.
73. Ohnishi S, et al. Effect of hypoxia on gene expression of bone marrow-derived mesenchymal stem cells and mononuclear cells. Stem Cells. 2007;25(5):1166–77.
74. Vandervelde S, et al. Signaling factors in stem cell-mediated repair of infarcted myocardium. J Mol Cell Cardiol. 2005;39(2):363–76.
75. Wang Y, et al. MEK mediates the novel cross talk between TNFR2 and TGF-EGFR in enhancing vascular endothelial growth factor (VEGF) secretion from human mesenchymal stem cells. Surgery. 2009;146(2):198–205.
76. Crisostomo PR, et al. Human mesenchymal stem cells stimulated by TNF-αlpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. Am J Physiol Cell Physiol. 2008;294(3):C675–82.
77. Tang YL, et al. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. Ann Thorac Surg. 2005;80(1):229–36. discussion 236–7.
78. Fan W, Crawford R, Xiao Y. The ratio of VEGF/PEDF expression in bone marrow mesenchymal stem cells regulates neovascularization. Differentiation. 2011;81(3):181–91.
79. Johansson U, et al. Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. Diabetes. 2008;57(9):2393–401.
80. Beckermann BM, et al. VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer. 2008;99(4):622–31.
81. da Silva Meirelles L, Chagastelles PC, Nardi NB. *Mesenchymal stem cells reside in virtually all post-natal organs and tissues*. J Cell Sci. 2006;119(Pt 11):2204–13.
82. Marian AJ, Roberts R. Familial hypertrophic cardiomyopathy: a paradigm of the cardiac hypertrophic response to injury. Ann Med. 1998;30(Suppl 1):24–32.
83. Zisa D, et al. Vascular endothelial growth factor (VEGF) as a key therapeutic trophic factor in bone marrow mesenchymal stem cell-mediated cardiac repair. Biochem Biophys Res Commun. 2009;390(3):834–8.
84. Urbich C, et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J Mol Cell Cardiol. 2005;39(5):733–42.
85. Shi M, Liu ZW, Wang FS. Immunomodulatory properties and therapeutic application of mesenchymal stem cells. Clin Exp Immunol. 2011;164(1):1–8.
86. Maccario R, et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica. 2005;90(4):516–25.
87. Zhang W, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev. 2004;13(3):263–71.
88. Spaggiari GM, et al. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006;107(4):1484–90.
89. Prigione I, et al. Reciprocal interactions between human mesenchymal stem cells and gammadelta T cells or invariant natural killer T cells. Stem Cells. 2009;27(3):693–702.
90. Nauta AJ, et al. Mesenchymal stem cells inhibit generation and function of both CD34+–derived and monocyte-derived dendritic cells. J Immunol. 2006;177(4):2080–7.
91. Corcione A, et al. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006;107(1):367–72.

92. Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. Annu Rev Pathol. 2011;6:457–78.
93. Kronsteiner B, et al. Human mesenchymal stem cells and renal tubular epithelial cells differentially influence monocyte-derived dendritic cell differentiation and maturation. Cell Immunol. 2011;267(1):30–8.
94. Gao S, et al. Mouse bone marrow-derived mesenchymal stem cells induce macrophage M2 polarization through the nuclear factor-kappaB and signal transducer and activator of transcription 3 pathways. Exp Biol Med (Maywood). 2014;239(3):366–75.
95. van den Berk LC, et al. Cord blood mesenchymal stem cells propel human dendritic cells to an intermediate maturation state and boost interleukin-12 production by mature dendritic cells. Immunology. 2009;128(4):564–72.
96. Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res. 2011;109(8):923–40.
97. Selmani Z, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells. 2008;26(1):212–22.
98. Krasnodembskaya A, et al. Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. Am J Physiol Lung Cell Mol Physiol. 2012;302(10):L1003–13.
99. Rasmusson I, et al. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation. 2003;76(8):1208–13.
100. Ren G, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2(2):141–50.
101. Sheng H, et al. A critical role of IFNgamma in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. Cell Res. 2008;18(8):846–57.
102. Gonzalez MA, et al. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum. 2009;60(4):1006–19.
103. Melief SM, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. Stem Cells. 2013;31(9):1980–91.
104. Deng W, et al. Effects of allogeneic bone marrow-derived mesenchymal stem cells on T and B lymphocytes from BXSB mice. DNA Cell Biol. 2005;24(7):458–63.
105. Krampera M, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells. 2006;24(2):386–98.
106. Bernardo ME, Locatelli F, Fibbe WE. Mesenchymal stromal cells. Ann N Y Acad Sci. 2009;1176:101–17.
107. Augello A, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2005;35(5):1482–90.
108. Sotiropoulou PA, et al. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells. 2006;24(1):74–85.
109. Le Blanc K, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363(9419):1439–41.
110. Li FR, et al. Immune modulation of co-transplantation mesenchymal stem cells with islet on T and dendritic cells. Clin Exp Immunol. 2010;161(2):357–63.
111. Di Ianni M, et al. Mesenchymal cells recruit and regulate T regulatory cells. Exp Hematol. 2008;36(3):309–18.
112. Djouad F, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood. 2003;102(10):3837–44.
113. Bassi EJ, Aita CA, Camara NO. Immune regulatory properties of multipotent mesenchymal stromal cells: where do we stand? World J Stem Cells. 2011;3(1):1–8.
114. Le Blanc K, et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol. 2003;31(10):890–6.

115. Nasef A, et al. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation. 2007;84(2):231–7.
116. Xu G, et al. Immunosuppressive properties of cloned bone marrow mesenchymal stem cells. Cell Res. 2007;17(3):240–8.
117. Ringden O, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006;81(10):1390–7.
118. Opitz CA, et al. Toll-like receptor engagement enhances the immunosuppressive properties of human bone marrow-derived mesenchymal stem cells by inducing indoleamine-2,3-dioxygenase-1 via interferon-beta and protein kinase R. Stem Cells. 2009;27(4):909–19.
119. Guo Z, et al. Mesenchymal stem cells reprogram host macrophages to attenuate obliterative bronchiolitis in murine orthotopic tracheal transplantation. Int Immunopharmacol. 2013;15(4):726–34.
120. Matsuda M, et al. Interleukin 10 pretreatment protects target cells from tumor- and Allo-specific cytotoxic T cells and downregulates HLA class I expression. J Exp Med. 1994;180(6):2371–6.
121. Petersson M, et al. Constitutive IL-10 production accounts for the high NK sensitivity, low MHC class I expression, and poor transporter associated with antigen processing (TAP)-1/2 function in the prototype NK target YAC-1. J Immunol. 1998;161(5):2099–105.
122. Bonder CS, et al. P-selectin can support both Th1 and Th2 lymphocyte rolling in the intestinal microvasculature. Am J Pathol. 2005;167(6):1647–60.
123. Ajuebor MN, et al. Role of resident peritoneal macrophages and mast cells in chemokine production and neutrophil migration in acute inflammation: evidence for an inhibitory loop involving endogenous IL-10. J Immunol. 1999;162(3):1685–91.
124. Nussler AK, et al. Leukocytes, the Janus cells in inflammatory disease. Langenbeck's Arch Surg. 1999;384(2):222–32.
125. Hegyi B, et al. Activated T-cells and pro-inflammatory cytokines differentially regulate prostaglandin E2 secretion by mesenchymal stem cells. Biochem Biophys Res Commun. 2012;419(2):215–20.
126. Yanez R, et al. Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. Exp Cell Res. 2010;316(19):3109–23.
127. Shinomiya S, et al. Regulation of TNFalpha and interleukin-10 production by prostaglandins I(2) and E(2): studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists. Biochem Pharmacol. 2001;61(9):1153–60.
128. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacol Ther. 2004;103(2):147–66.
129. Kang JW, et al. Immunomodulatory effects of human amniotic membrane-derived mesenchymal stem cells. J Vet Sci. 2012;13(1):23–31.
130. Hill M, et al. IDO expands human CD4+CD25high regulatory T cells by promoting maturation of LPS-treated dendritic cells. Eur J Immunol. 2007;37(11):3054–62.
131. Kahler DJ, Mellor AL. T cell regulatory plasmacytoid dendritic cells expressing indoleamine 2,3 dioxygenase. Handb Exp Pharmacol. 2009;188:165–96.
132. Terness P, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med. 2002;196(4):447–57.
133. Ryan JM, et al. Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol. 2007;149(2):353–63.
134. Frumento G, et al. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med. 2002;196(4):459–68.

Stem Cell-Based Therapies for Acute Lung Injury and Acute Respiratory Distress Syndrome

18

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Abstract

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) characterized by severe inflammation and lung injury causes high morbidity and mortality. Currently, there have been no effective therapies for ALI/ARDS. Stem cells, with multipotent capacity the potential therapeutic option for ALI/ARDS by modulating the immune response and promoting repair of the damaged tissue. Of the various stem cell-based therapies, mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs), stem-like cells, stem cell-educated cells, and modified stem cells have the accumulated data to support their potential therapeutic efficacy for lung injury. Stem cells appear to exert their effects via multiple mechanisms. Recently, the release of paracrine factors microvesicles and/or exosome has been shown to correlate with stem cell-afforded protection against ALI. Encouragingly, two early-phase clinical trials of stem cells in patients with ARDS demonstrated their safety with no serious adverse effects. Nevertheless, stem cell-based therapies offer both opportunities and challenges for the treatment of ARDS patients. Moreover, the identified standard in order to choose ARDS patients for stem cell-based therapy and longer-term adverse effects under stem cell-based therapy must be established and evaluated. In this section, we aimed to emphasize that stem cells are a highly promising potential therapy for ALI/ARDS.

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18.1 Acute Lung Injury (ALI)/Acute Respiratory Distress Syndrome (ARDS)

18.1.1 Epidemiology of ALI/ARDS

The acute lung injury (ALI) and its most severe form, acute respiratory distress syndrome (ARDS), are manifested by acute onset, bilateral lung infiltrates, noncardiogenic respiratory failure, and progressive hypoxemia as defined by the ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen, PaO_2 $\leq$300 mmHg [1–3]. ALI/ARDS is characterized by increased permeability of the alveolar barrier, flooded airspaces with protein-rich edema fluid, and impaired arterial oxidation [4]. There are many causes of ALI/ARDS, including sepsis, pneumonia, trauma, pancreatitis, or blood transfusion [5]. Sepsis is the most important cause of ALI/ARDS. The incidence of ALI/ARDS is about 86.2 per 100,000 population worldwide [6]. Although new treatments are being applied, the mortality rate of ALI/ARDS remains as high as 26–35%, depending on various factors such as comorbidities [7]. Therefore, the prevention and treatment of ALI/ARDS are still the urgent problem to be solved.

18.1.2 Pathogenesis of ALI/ARDS

In general, ALI/ARDS is a highly complicated disease process. Earlier concepts on the progression of ALI/ARDS are from early "exudative phase" to later "fibroproliferative phase" [8]. In the exudative phase of ALI/ARDS, the type I alveolar epithelial cells (AECs) and capillary endothelium cells are particularly susceptible to injury caused by direct or indirect insults [9]. As a result, impaired epithelium is replaced by the formation of proteinaceous hyaline membranes. As following, numerous circulating neutrophils migrate into the lung [8]. It has also been well documented that the activated neutrophils release a variety of injurious molecules including neutrophil elastase, metalloproteases, proteolytic enzymes, oxidants, and reactive oxygen species [10]. In addition, the sequestration of neutrophils is mediated by chemoattractant factors and by the adhesion molecules expressed on both neutrophils and capillary endothelial cells [11]. Thus, neutrophil activation, migration, and sequestration are the characteristic events in the early progression of ALI/ARDS.

In addition to the exudative phase, excessive fibroblasts, myofibroblasts, and pluripotential mesenchymal progenitor cells gather around the pulmonary alveolar space in the fibroproliferative phase of ARDS [12]. In this stage of ARDS, apoptosis and necrosis are often observed in the type I AECs, which is quickly replaced by the type II AECs. Consequently, new blood vessels form within the provisional matrix. More importantly, the mortality rate is high in patients who are under fibroproliferative response of ALI/ARDS [13].

18.1.3 The Therapeutic Challenge of ALI/ARDS

Up to now, it has been difficult to plan a unified treatment strategy for patients with ALI/ARDS due to several reasons. Firstly, the diagnosis of ALI/ARDS is somewhat difficult. To diagnose a patient with ALI/ARDS, a set of clinical parameters must be satisfied, which include hypoxia and bilateral infiltrates on chest X-ray in the absence of left atrial hypertension in order to rule out cardiac failure. The ARDS clinical trial network has looked at the biologic index of biomarkers combined with clinical risk factors, indicating that it is most unlikely that any one single biomarker is capable of precisely and accurately predicting the risk of development, diagnosis, and prognostic course of ARDS in patients. Secondly, the patient population with ALI/ARDS is heterogeneous. There are significant differences in age, general health status, and the cause of ALI/ARDS. It is hard to determine if ALI/ARDS is a single disease or a complication of different disease processes with a similar phenotype. To this end, heterogeneity in different patients increases the difficulty for treatment of ALI/ARDS. Thirdly, the understanding of the pathogenesis of ALI/ARDS remains limited. In addition to the earlier concept of distinct two disease phases, we now accept that the "early phase" and "later phase" of ALI/ARDS largely coexist. The pro-inflammatory response, impaired immune response, and repairing and fibrosis stage may all present in the complex milieu of ALI/ARDS.

Given these complexities, many different therapies for ALI/ARDS have been evaluated including antioxidants [14], surfactant [15], nitric oxide [16], corticosteroids [17–19], immunomodulatory agents [20, 21], and beta-2 agonists [22]. But there are no effective pharmacologic treatments for ALI/ARDS. Recently, stem cell-based therapies offer considerable promise for the treatment of ALI/ARDS [23, 24].

18.2 Why Stem Cells Might Be a Candidate for Treating ALI/ARSD

Stem cells can divide asymmetrically to produce another cell-like themselves or more differentiated cells. Stem cells are classified based on their tissue of origin. Embryonic stem cells (ESCs) are derived from the inner blastocyst cell mass, while adult tissue-derived stem cells include mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and endogenous lung stem cells [25]. Stem cells are multipotent and have the potential to differentiate into a more limited range of mature cell types.

Even just based on the biological characteristics of stem cells, there are a number of reasons to support that stem cells can be used as a therapy for ALI/ARDS (Fig. 18.1). First, stem cells can be migrated and homed in conjunction with the involvement of chemoattractant cytokines, proteolytic enzymes, and surface adhesion molecules. Stem cells, especially MSCs, are able to interact with pulmonary endothelial cells directly and maintain the integrity of endothelial barrier by

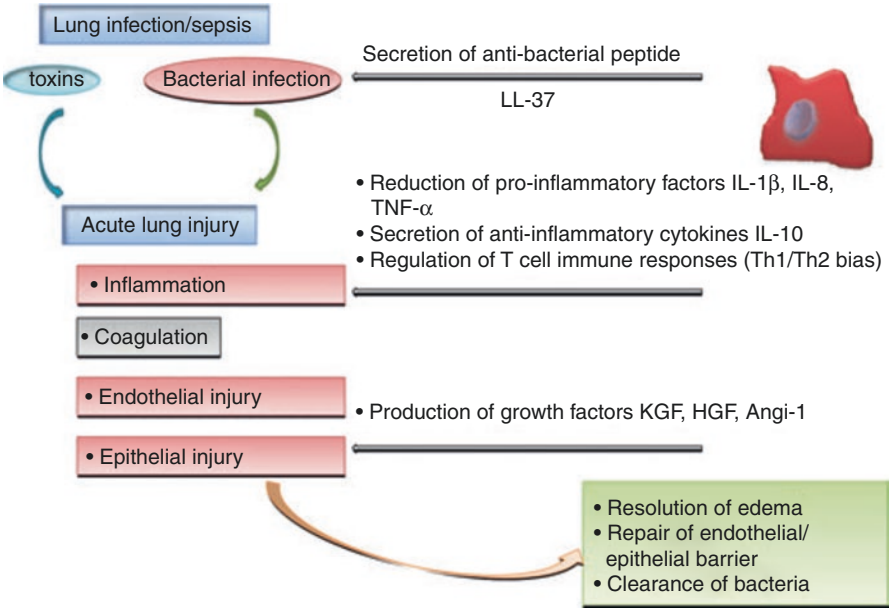

Fig. 18.1 A promising way of stem cell-based cell therapy in ALI/ARDS (Reproduced with permission from Xu et al. [26])

preserving adherent junctions and inhibiting leukocyte adhesion and adhesion molecule expression [27]. Second, stem cells can protect the host from extraordinary inflammatory damage by enhancing host resistance to sepsis; downregulating expressions of pro-inflammatory cytokines such as IL-1β, IL-8, and TNF-a [25]; and upregulating production of anti-inflammatory cytokines such as IL-4 and IL-10 [28]. Third, stem cells suppress and/or modulate innate and adaptive immune responses [29]. Stem cells show their ability to switch Th1 cell-based inflammatory response to Th2 cell-based anti-inflammatory response during the inflammation. Therefore, stem cells have more complicated immunomodulatory effects. Fourth, treatment with stem cells is capable of attenuating pulmonary edema in ALI [30]. On the one hand, stem cells produce several growth factors including keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) to reduce pulmonary edema. On the other hand, implanted stem cells repair alveolar-capillary membrane, reduce alveolar-capillary membrane permeability, and improve diffused pulmonary edema [31]. Fifth, stem cells might directly reduce bacterial burden by enhancing the phagocytosis of resident immune cells, increasing bacterial clearance [32], and secreting antimicrobial peptide [33]. Finally, stem cells can differentiate into lung cells, thus directly replacing damaged cells during ALI/ARDS.

18.3 The Stem Cell-Based Therapies for ALI/ARDS

18.3.1 Mesenchymal Stem Cells (MSCs)

MSCs are multipotent adult progenitor cells that can be isolated from bone marrow, umbilical cord, umbilical cord blood, adipose tissue, placenta, and many others [34]. Besides, MSCs can be expanded to large numbers ex vivo and employed in a variety of applications including in the treatment of cardiovascular diseases, pulmonary fibrosis, spinal cord injury, bone repair, and cartilage repair [35]. Moreover, MSCs express high negative costimulatory molecules (B7-H1, B7-H3, and B7-H4), but no class II major histocompatibility complex (MHC-II) antigens, which allow them to have the advantage of low immunogenicity [36, 37]. Therefore, MSCs have been one of the most widely studied stem/progenitor cells for cell-based therapy [38].

At first, there were three independent studies on using bone marrow-derived MSCs (BM-MSCs) in experimental models of ALI/ARDS in 2007 [39–41]. All three reports showed that treatment with BM-MSCs prevented LPS-induced ALI by reducing LPS-induced cellular and humoral inflammation, pulmonary edema, and neutrophil recruitment. More recently, accumulated evidence further demonstrated the protection afforded by administration of BM-MSCs against ALI induced by either endotoxin or live *Escherichia coli* (*E. coli*) bacteria [33, 42, 43], or following sepsis [32, 44]. While BM-MSCs were used in most of the reported studies, promising results have also been reported using MSCs derived from alternative sources including umbilical cord (UC-MSCs), umbilical cord blood (UCB-MSCs), and adipose tissue (AD-MSCs). Li and colleagues found that UC-MSCs noticeably increased the survival rate of rats subjected to LPS-induced ALI and significantly reduced systemic and pulmonary inflammation, probably by promoting anti-inflammatory homeostasis and reducing oxidative stress [23]. Lee et al. further reported that early therapy using UC-MSCs reduced mortality in a rat model of ARDS complicated by sepsis [45]. UCB-MSCs also ameliorated *E. coli*-induced ALI in mice [46]. Of note, there was a clinical application of UCB-MSCs therapy in a patient with ARDS reported [47]. Moreover, AD-MSCs transplantation was also shown to be effective in modulating inflammation during ALI by inhibiting the acute inflammatory response [48].

18.3.2 Endothelial Progenitor Cells (EPCs)

In 1997, Asahara initially described and defined EPCs as a special type of stem cells [49]. EPCs have the capacity to proliferate, migrate, and differentiate into endothelial cells (ECs), while no characteristics of mature ECs. EPCs can be isolated from bone marrow, spleen, umbilical cord blood, and peripheral blood. Some studies have proved that EPCs can engineer new blood vessels in postnatal life and differentiate into mature endothelial cells in the injured vessels [50, 51].

During ALI/ARDS, diffused endothelial cells apoptosis and necrosis can cause severe lung damage and high mortality [52]. Once endothelial cells die, the lost function of these dead endothelial cells may be compensated by the mature endothelial cells from nearby tissues [53]. However, mature endothelial cells have a low proliferative potential, and their capacity to replace damaged endothelial cells and to create new vessels is therefore limited. Now, emerged evidences suggest that EPCs released into the peripheral blood are able to differentiate into and replace dead endothelial cells due to their high proliferative potential compared to mature endothelial cells in nearby tissues [54, 55]. Besides, there is a dose-dependent relationship between the degree of lung injury and the amount of EPCs released from the bone marrow [56]. Altogether, these studies suggest that EPCs can migrate into the injured lung tissue, maintain the integrity of pulmonary alveolar-capillary barrier, reestablish the endothelial function in vessels, and ameliorate inflammation [57–59]. The underlying mechanism(s) by which transplanted EPCs ameliorate ALI is not fully clarified; however, several factors targeted by EPCs have been implicated, including EPC-induced decrease in adhesion molecule ICAM-1 and P-selectin expression [60], inhibition of pulmonary microvascular endothelial cell apoptosis [61], prevention of increased pulmonary vascular permeability [62], and downregulation of pro-inflammatory cytokines TNF-α, IFN-γ, and IL-1β, while upregulation of anti-inflammatory cytokine IL-10 [59, 60, 63].

18.3.3 Other Stem-Like Cells and Stem Cell-Educated Cells

Although MSCs and EPCs-based therapy is a promising therapeutic strategy for ALI/ARDS, other stem-like cells or stem cell-educated cells have also been intelligible in treating ALI/ARDS. Maron-Gutierrez and colleagues demonstrated the beneficial effects of bone marrow-derived mononuclear cells (BMDMCs) in ALI/ARDS in terms of lung mechanics, lung inflammation, and mortality [64]. Administration of BMDMCs mitigated pulmonary inflammation, decreased lung elastance, and attenuated lung remodeling and fibrosis, thus resulting in an improved survival in a murine model of ALI/ARDS [31, 65, 66]. Therefore, the benefits of BMDMCs depend on the type of initial insult as well as their different effects on endothelial cell activation and adhesion molecules. A separate study further demonstrated the therapeutic benefit of $CD34^+$ cells isolated from human umbilical cord blood, providing evidence of the potential of cord blood progenitor cells for vascular repair in ARDS [65]. Our previous work also showed that human amniotic fluid stem (hAFS) cells as an important type of stem cells displayed remarkable positive effects on ALI-damaged lung tissue repair, which include recovery of the integrity of alveolar-capillary membrane, attenuation of leukocyte and neutrophil transepithelial migration, and downregulation of pro-inflammatory cytokine and chemokine expression [67]. Finally, other stem-like cells isolated from dental pulp, menstrual blood-derived stem cells (MenSCs), and even educated macrophages by MSCs have also been investigated in preclinical models of ALI [68–70]. Altogether, these studies further suggest that there may be alternative sources of cells with potentials to treat ARDS in future.

18.4 Modified Stem Cell Therapy for ALI/ARDS

An earlier study has demonstrated that gene overexpression strategies can be used to enhance MSC efficacy, specifically overexpression of Ang-1 [40]. Afterward, MSCs overexpressing soluble IL-1 receptor-like-1, which plays an immune-modulatory role in the lung, decreased inflammation, BAL protein, and neutrophil content compared to naive MSCs in a mouse model of LPS-induced ALI [71]. Recently, administration of BM-MSCs expressing keratinocyte growth factor (KGF) in the lung induced proliferation of lung epithelial cells and promoted the secretion of surface proteins [72]. In addition to KGF, hepatocyte growth factor (HGF) is also an important cytokine in the cell and tissue repairment and regeneration. It is proven that HGF-modified mesenchymal stem cells improved ischemia/reperfusion-induced ALI in rats [73]. Furthermore, MSCs overexpressing angiotensin-converting enzyme 2 (ACE2) showed enhanced endothelial repair and inhibited expression of pro-inflammatory cytokines including TNF-α and IL-6 following LPS challenge when compared with naive MSCs [74]. Chemokine-induced mobilization of inflammatory cells contributes predominantly to inflammation and lung tissue damage in the pathogenesis of ALI/ARDS. Yang et al. demonstrated that overexpression of CXCR4 in MSCs enhanced therapeutic potential of MSCs in the treatment of ALI [75]. Finally, a recent study focused on the nuclear factor erythroid 2-related factor 2 (Nrf2) which is a key transcription factor and plays a central role in inducible expression of many cytoprotective proteins in response to oxidative and electrophilic stress. Nrf2-overexpressed human amniotic mesenchymal stem cells (hAMSCs) exhibited increased cell retention in the lung, more efficient differentiation into AT II cells, and more prominent effects on the increased mRNA and protein expression as well as DNA-binding activity of Nrf2 than naïve hAMSCs [76].

18.5 Stem Cell-Derived Microvesicles Might Be Useful in ALI/ARDS

Cell-based therapy with stem cells is an attractive therapeutic approach. Recently, MSCs have also been found to release circular membrane fragments called microparticles or microvesicles (MVs), which are involved in cell-cell communication and the transfer of cellular materials [77]. Subsequent studies suggested that the therapeutic effect of MSC-derived MVs in kidney injury was through the transfer of mRNA and miRNA to the injured renal epithelium [78, 79], leading to a decrease in cell apoptosis. One study found that human MSC-derived MVs were therapeutically effective in a murine model of LPS-induced ALI, partly through the enhanced expression of KGF mRNA in the injured alveolus [24]. Another study observed that human MSC-derived MVs improved survival in a mouse model of severe pneumonia [80]. A subsequent study instituted that exosome from induced pluripotent stem cells (iPSCs) delivering siRNA attenuated intracellular adhesion molecule-1 expression and neutrophils adhesion in pulmonary microvascular endothelial cells [81]. Tang et al. further reported that MVs derived from human mesenchymal stem cells ameliorated inflammation in the lungs, which was partly mediated by Ang-1 mRNA [82].

18.6 Clinical Translation and Prospective Strategies

Based largely on numerous preclinical studies, administration of mesenchymal stem or stromal cells (MSCs) as a therapeutic for ALI/ARDS holds great promise, and clinical trials are currently underway. Recently, two research teams have proceeded to early-phase clinical testing of stem cell therapy for patients with ALI/ARDS. In one phase I trial, Zhang et al. demonstrated that a low dose of 1×10^6 adipose-derived MSCs per kg body weight was well tolerated in 12 ARDS patients (NCT01902082) [83]. They evaluated the safety and feasibility of intravenously infused fresh allogeneic MSCs and concluded that higher doses of MSCs may be needed in the phase II stage. In another phase I trial, Wilson et al. tested three doses of human bone marrow-derived MSCs at 1×10^6, 5×10^6, and 10×10^6 MSCs per kg body weight given intravenously in nine patients with moderate to severe ARDS (NCT01775774) [84]. As a result, there were no adverse effects seen at any of the doses used. With the favorable safety profile of MSCs in phase I trial, this research group is now conducting a randomized, blinded phase II safety trial in 60 patients (NCT02097641).

Although MSCs demonstrate considerable promise and early-phase clinical trials are encouraging, the future clinical translation on MSCs for patients with ARDS is still challenging. First, the type and potency of stem cells must be considered. Second, ARDS is a clinical syndrome rather than a disease; stem cell-based therapy may be not useful for all patients. Therefore, we need an identified standard to choose appropriate ARDS patients for stem cell-based therapy. Third, the doses, administration route, and timing for treatment remain to be determined. Finally, longer-term adverse effects in patients with ARDS under stem cell-based therapy also need to be evaluated. Nevertheless, stem cell-based therapies offer promise and issues for the treatment of ARDS in the clinic.

In conclusion, stem cells are a promising therapy for patients suffering from ALI/ARDS. Stem cell-based therapy favorably modulates the immune response to reduce lung injury both in experiment animal models of ALI/ARDS and in patients with ALI/ARDS. Furthermore, the phase I clinical trials have shown that stem-cell based therapy is safe. Nevertheless, stem cell-based therapy for ALI/ARDS offers both opportunities and challenges. We anticipate that the abovementioned problems will be solved by further studies. Ultimately, the clinical translation of stem cell-based therapy from preclinical studies to clinical trials for treatment of patients with ALI/ARDS will achieved in the near future.

References

1. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012;307:2526–33.
2. Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. J Clin Invest. 2012;122:2731–40.

3. Wheeler AP, Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. Lancet. 2007;369:1553–64.
4. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342:1334–49.
5. Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med. 2006;27:337–49.
6. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European consensus conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994;149:818–24.
7. Erickson SE, Martin GS, Davis JL, Matthay MA, Eisner MD, Network NNA. Recent trends in acute lung injury mortality: 1996-2005. Crit Care Med. 2009;37:1574–9.
8. Standiford TJ, Ward PA. Therapeutic targeting of acute lung injury and acute respiratory distress syndrome. Transl Res. 2016;167:183–91.
9. Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. Clin Chest Med. 1982;3:35–56.
10. Abraham E. Neutrophils and acute lung injury. Crit Care Med. 2003;31:S195–9.
11. Hasko G, Xu DZ, Lu Q, Nemeth ZH, Jabush J, Berezina TL, Zaets SB, Csoka B, Deitch EA. Adenosine A2A receptor activation reduces lung injury in trauma/hemorrhagic shock. Crit Care Med. 2006;34:1119–25.
12. Chesnutt AN, Matthay MA, Tibayan FA, Clark JG. Early detection of type III procollagen peptide in acute lung injury. Pathogenetic and prognostic significance. Am J Respir Crit Care Med. 1997;156:840–5.
13. Martin C, Papazian L, Payan MJ, Saux P, Gouin F. Pulmonary fibrosis correlates with outcome in adult respiratory distress syndrome. A study in mechanically ventilated patients. Chest. 1995;107:196–200.
14. Bernard GR, Wheeler AP, Arons MM, Morris PE, Paz HL, Russell JA, Wright PE. A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The antioxidant in ARDS study group. Chest. 1997;112:164–72.
15. Kesecioglu J, Beale R, Stewart TE, Findlay GP, Rouby JJ, Holzapfel L, Bruins P, Steenken EJ, Jeppesen OK, Lachmann B. Exogenous natural surfactant for treatment of acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med. 2009;180:989–94.
16. Taut FJ, Rippin G, Schenk P, Findlay G, Wurst W, Hafner D, Lewis JF, Seeger W, Gunther A, Spragg RG. A search for subgroups of patients with ARDS who may benefit from surfactant replacement therapy: a pooled analysis of five studies with recombinant surfactant protein-C surfactant (Venticute). Chest. 2008;134:724–32.
17. Meduri GU, Marik PE, Chrousos GP, Pastores SM, Arlt W, Beishuizen A, Bokhari F, Zaloga G, Annane D. Steroid treatment in ARDS: a critical appraisal of the ARDS network trial and the recent literature. Intensive Care Med. 2008;34:61–9.
18. Tang BM, Craig JC, Eslick GD, Seppelt I, McLean AS. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med. 2009;37:1594–603.
19. Thompson BT. Glucocorticoids and acute lung injury. Crit Care Med. 2003;31:S253–7.
20. Iwata K, Doi A, Ohji G, Oka H, Oba Y, Takimoto K, Igarashi W, Gremillion DH, Shimada T. Effect of neutrophil elastase inhibitor (sivelestat sodium) in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): a systematic review and meta-analysis. Intern Med. 2010;49:2423–32.
21. Presneill JJ, Harris T, Stewart AG, Cade JF, Wilson JW. A randomized phase II trial of granulocyte-macrophage colony-stimulating factor therapy in severe sepsis with respiratory dysfunction. Am J Respir Crit Care Med. 2002;166:138–43.
22. Matthay MA, Brower RG, Carson S, Douglas IS, Eisner M, Hite D, Holets S, Kallet RH, Liu KD, MacIntyre N, et al. Randomized, placebo-controlled clinical trial of an aerosolized beta(2)-agonist for treatment of acute lung injury. Am J Respir Crit Care Med. 2011;184:561–8.

23. Li J, Li D, Liu X, Tang S, Wei F. Human umbilical cord mesenchymal stem cells reduce systemic inflammation and attenuate LPS-induced acute lung injury in rats. J Inflamm (Lond). 2012;9:33.
24. Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, Qu JM, Matthay MA, Lee JW. Human mesenchymal stem cell microvesicles for treatment of *Escherichia coli* endotoxin-induced acute lung injury in mice. Stem Cells. 2014;32:116–25.
25. Hayes M, Curley G, Ansari B, Laffey JG. Clinical review: stem cell therapies for acute lung injury/acute respiratory distress syndrome - hope or hype? Crit Care. 2012;16:205.
26. Xu F, Hu Y, Zhou J, Wang X. Mesenchymal stem cells in acute lung injury: are they ready for translational medicine? J Cell Mol Med. 2013;17(8):927–35.
27. Pati S, Gerber MH, Menge TD, Wataha KA, Zhao Y, Baumgartner JA, Zhao J, Letourneau PA, Huby MP, Baer LA, et al. Bone marrow derived mesenchymal stem cells inhibit inflammation and preserve vascular endothelial integrity in the lungs after hemorrhagic shock. PLoS One. 2011;6:e25171.
28. Liang ZX, Sun JP, Wang P, Tian Q, Yang Z, Chen LA. Bone marrow-derived mesenchymal stem cells protect rats from endotoxin-induced acute lung injury. Chin Med J. 2011;124:2715–22.
29. Frank MH, Sayegh MH. Immunomodulatory functions of mesenchymal stem cells. Lancet. 2004;363:1411–2.
30. Folkesson HG, Matthay MA. Alveolar epithelial ion and fluid transport: recent progress. Am J Respir Cell Mol Biol. 2006;35:10–9.
31. Prota LF, Lassance RM, Maron-Gutierrez T, Castiglione RC, Garcia CS, Santana MC, Souza-Menezes J, Abreu SC, Samoto V, Santiago MF, et al. Bone marrow mononuclear cell therapy led to alveolar-capillary membrane repair, improving lung mechanics in endotoxin-induced acute lung injury. Cell Transplant. 2010;19:965–71.
32. Mei SH, Haitsma JJ, Dos Santos CC, Deng Y, Lai PF, Slutsky AS, Liles WC, Stewart DJ. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. Am J Respir Crit Care Med. 2010;182:1047–57.
33. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells. 2010;28:2229–38.
34. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. Cell Transplant. 2011;20:5–14.
35. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol. 2004;36:568–84.
36. Devine SM, Hoffman R. Role of mesenchymal stem cells in hematopoietic stem cell transplantation. Curr Opin Hematol. 2000;7:358–63.
37. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond). 2005;2:8.
38. Prockop DJ, Gregory CA, Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci U S A. 2003;100(Suppl 1):11917–23.
39. Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol. 2007;179:1855–63.
40. Mei SH, McCarter SD, Deng Y, Parker CH, Liles WC, Stewart DJ. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. PLoS Med. 2007;4:e269.
41. Xu J, Woods CR, Mora AL, Joodi R, Brigham KL, Iyer S, Rojas M. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. Am J Physiol Lung Cell Mol Physiol. 2007;293:L131–41.
42. Gupta N, Krasnodembskaya A, Kapetanaki M, Mouded M, Tan X, Serikov V, Matthay MA. Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. Thorax. 2012;67:533–9.
43. Lee JW, Krasnodembskaya A, McKenna DH, Song Y, Abbott J, Matthay MA. Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. Am J Respir Crit Care Med. 2013;187:751–60.

44. Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15:42–9.
45. Lee FY, Chen KH, Wallace CG, Sung PH, Sheu JJ, Chung SY, Chen YL, Lu HI, Ko SF, Sun CK, et al. Xenogeneic human umbilical cord-derived mesenchymal stem cells reduce mortality in rats with acute respiratory distress syndrome complicated by sepsis. Oncotarget. 2017;8:45626–42.
46. Kim ES, Chang YS, Choi SJ, Kim JK, Yoo HS, Ahn SY, Sung DK, Kim SY, Park YR, Park WS. Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells attenuates *Escherichia coli*-induced acute lung injury in mice. Respir Res. 2011;12:108.
47. Chang Y, Park SH, Huh JW, Lim CM, Koh Y, Hong SB. Intratracheal administration of umbilical cord blood-derived mesenchymal stem cells in a patient with acute respiratory distress syndrome. J Korean Med Sci. 2014;29:438–40.
48. Chien MH, Bien MY, Ku CC, Chang YC, Pao HY, Yang YL, Hsiao M, Chen CL, Ho JH. Systemic human orbital fat-derived stem/stromal cell transplantation ameliorates acute inflammation in lipopolysaccharide-induced acute lung injury. Crit Care Med. 2012;40:1245–53.
49. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964–7.
50. Watt SM, Athanassopoulos A, Harris AL, Tsaknakis G. Human endothelial stem/progenitor cells, angiogenic factors and vascular repair. J R Soc Interface. 2010;7(Suppl 6):S731–51.
51. Zampetaki A, Kirton JP, Xu Q. Vascular repair by endothelial progenitor cells. Cardiovasc Res. 2008;78:413–21.
52. Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K. Acute lung injury review. Intern Med. 2009;48:621–30.
53. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell. 2005;121:823–35.
54. Bompais H, Chagraoui J, Canron X, Crisan M, Liu XH, Anjo A, Tolla-Le Port C, Leboeuf M, Charbord P, Bikfalvi A, Uzan G. Human endothelial cells derived from circulating progenitors display specific functional properties compared with mature vessel wall endothelial cells. Blood. 2004;103:2577–84.
55. Kahler CM, Wechselberger J, Hilbe W, Gschwendtner A, Colleselli D, Niederegger H, Boneberg EM, Spizzo G, Wendel A, Gunsilius E, et al. Peripheral infusion of rat bone marrow derived endothelial progenitor cells leads to homing in acute lung injury. Respir Res. 2007;8:50.
56. Yamada M, Kubo H, Ishizawa K, Kobayashi S, Shinkawa M, Sasaki H. Increased circulating endothelial progenitor cells in patients with bacterial pneumonia: evidence that bone marrow derived cells contribute to lung repair. Thorax. 2005;60:410–3.
57. He T, Peterson TE, Holmuhamedov EL, Terzic A, Caplice NM, Oberley LW, Katusic ZS. Human endothelial progenitor cells tolerate oxidative stress due to intrinsically high expression of manganese superoxide dismutase. Arterioscler Thromb Vasc Biol. 2004;24:2021–7.
58. Lam CF, Liu YC, Hsu JK, Yeh PA, Su TY, Huang CC, Lin MW, Wu PC, Chang PJ, Tsai YC. Autologous transplantation of endothelial progenitor cells attenuates acute lung injury in rabbits. Anesthesiology. 2008;108:392–401.
59. Mao M, Wang SN, Lv XJ, Wang Y, Xu JC. Intravenous delivery of bone marrow-derived endothelial progenitor cells improves survival and attenuates lipopolysaccharide-induced lung injury in rats. Shock. 2010;34:196–204.
60. Cao JP, He XY, Xu HT, Zou Z, Shi XY. Autologous transplantation of peripheral blood-derived circulating endothelial progenitor cells attenuates endotoxin-induced acute lung injury in rabbits by direct endothelial repair and indirect immunomodulation. Anesthesiology. 2012;116:1278–87.

61. Xia L, Fu GS, Yang JX, Zhang FR, Wang XX. Endothelial progenitor cells may inhibit apoptosis of pulmonary microvascular endothelial cells: new insights into cell therapy for pulmonary arterial hypertension. Cytotherapy. 2009;11:492–502.
62. Wary KK, Vogel SM, Garrean S, Zhao YD, Malik AB. Requirement of alpha(4)beta(1) and alpha(5)beta(1) integrin expression in bone-marrow-derived progenitor cells in preventing endotoxin-induced lung vascular injury and edema in mice. Stem Cells. 2009;27:3112–20.
63. Toya SP, Li F, Bonini MG, Gomez I, Mao M, Bachmaier KW, Malik AB. Interaction of a specific population of human embryonic stem cell-derived progenitor cells with CD11b+ cells ameliorates sepsis-induced lung inflammatory injury. Am J Pathol. 2011;178:313–24.
64. Maron-Gutierrez T, Silva JD, Cruz FF, Alegria S, Xisto DG, Assis EF, Castro-Faria-Neto HC, Dos Santos CC, Morales MM, Rocco PR. Insult-dependent effect of bone marrow cell therapy on inflammatory response in a murine model of extrapulmonary acute respiratory distress syndrome. Stem Cell Res Ther. 2013;4:123.
65. Araujo IM, Abreu SC, Maron-Gutierrez T, Cruz F, Fujisaki L, Carreira H Jr, Ornellas F, Ornellas D, Vieira-de-Abreu A, Castro-Faria-Neto HC, et al. Bone marrow-derived mononuclear cell therapy in experimental pulmonary and extrapulmonary acute lung injury. Crit Care Med. 2010;38:1733–41.
66. Ornellas DS, Maron-Gutierrez T, Ornellas FM, Cruz FF, Oliveira GP, Lucas IH, Fujisaki L, Oliveira MG, Teodoro WR, Capelozzi VL, et al. Early and late effects of bone marrow-derived mononuclear cell therapy on lung and distal organs in experimental sepsis. Respir Physiol Neurobiol. 2011;178:304–14.
67. Xu Y, Xiang J, Zhao H, Liang H, Huang J, Li Y, Pan J, Zhou H, Zhang X, Wang JH, et al. Human amniotic fluid stem cells labeled with up-conversion nanoparticles for imaging-monitored repairing of acute lung injury. Biomaterials. 2016;100:91–100.
68. Hu Y, Qin C, Zheng G, Lai D, Tao H, Zhang Y, Qiu G, Ge M, Huang L, Chen L, et al. Mesenchymal stem cell-educated macrophages ameliorate LPS-induced systemic response. Mediat Inflamm. 2016;2016:3735452.
69. Wakayama H, Hashimoto N, Matsushita Y, Matsubara K, Yamamoto N, Hasegawa Y, Ueda M, Yamamoto A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating acute lung injury in mice. Cytotherapy. 2015;17:1119–29.
70. Xiang B, Chen L, Wang X, Zhao Y, Wang Y, Xiang C. Transplantation of menstrual blood-derived mesenchymal stem cells promotes the repair of LPS-induced acute lung injury. Int J Mol Sci. 2017;18:689.
71. Martinez-Gonzalez I, Roca O, Masclans JR, Moreno R, Salcedo MT, Baekelandt V, Cruz MJ, Rello J, Aran JM. Human mesenchymal stem cells overexpressing the IL-33 antagonist soluble IL-1 receptor-like-1 attenuate endotoxin-induced acute lung injury. Am J Respir Cell Mol Biol. 2013;49:552–62.
72. Chen J, Li C, Gao X, Li C, Liang Z, Yu L, Li Y, Xiao X, Chen L. Keratinocyte growth factor gene delivery via mesenchymal stem cells protects against lipopolysaccharide-induced acute lung injury in mice. PLoS One. 2013;8:e83303.
73. Chen S, Chen X, Wu X, Wei S, Han W, Lin J, Kang M, Chen L. Hepatocyte growth factor-modified mesenchymal stem cells improve ischemia/reperfusion-induced acute lung injury in rats. Gene Ther. 2017;24:3–11.
74. He HL, Liu L, Chen QH, Cai SX, Han JB, Hu SL, Chun P, Yang Y, Guo FM, Huang YZ, Qiu HB. MSCs modified with ACE2 restore endothelial function following LPS challenge by inhibiting the activation of RAS. J Cell Physiol. 2015;230:691–701.
75. Yang JX, Zhang N, Wang HW, Gao P, Yang QP, Wen QP. CXCR4 receptor overexpression in mesenchymal stem cells facilitates treatment of acute lung injury in rats. J Biol Chem. 2015;290:1994–2006.
76. Zhang S, Jiang W, Ma L, Liu Y, Zhang X, Wang S. Nrf2 transfection enhances the efficacy of human amniotic mesenchymal stem cells to repair lung injury induced by lipopolysaccharide. J Cell Biochem. 2017;19(2):1627–36.
77. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011;68:2667–88.

78. Bruno S, Grange C, Collino F, Deregibus MC, Cantaluppi V, Biancone L, Tetta C, Camussi G. Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. PLoS One. 2012;7:e33115.
79. Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C, Camussi G. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant. 2011;26:1474–83.
80. Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, Rosenzwajg M, Matthay MA, Lee JW. Therapeutic effects of human mesenchymal stem cell-derived microvesicles in severe pneumonia in mice. Am J Respir Crit Care Med. 2015;192:324–36.
81. Ju Z, Ma J, Wang C, Yu J, Qiao Y, Hei F. Exosomes from iPSCs delivering siRNA attenuate intracellular adhesion Molecule-1 expression and neutrophils adhesion in pulmonary microvascular endothelial cells. Inflammation. 2017;40:486–96.
82. Tang XD, Shi L, Monsel A, Li XY, Zhu HL, Zhu YG, Qu JM. Mesenchymal stem cell microvesicles attenuate acute lung injury in mice partly mediated by Ang-1 mRNA. Stem Cells. 2017;35:1849–59.
83. Zheng G, Huang L, Tong H, Shu Q, Hu Y, Ge M, Deng K, Zhang L, Zou B, Cheng B, Xu J. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. Respir Res. 2014;15:39.
84. Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, Cosgrove K, Vojnik R, Calfee CS, Lee JW, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respir Med. 2015;3:24–32.

Advanced Techniques in Burn Wound Repair

19

Jianglin Tan and Gaoxing Luo

Abstract

In order to facilitate the rate and quality of burn wound healing, many advanced techniques emerged in recent years, which contribute to the improved level of diagnosis and treatment of burn injury. Precise diagnosis; early, modified cooling therapy; and surgical intervention for burn wound healing are discussed firstly in this review. Then some new methods or materials such as modern wound dressing, negative pressure wound therapy, phototherapy, ultrasound therapy, platelet-rich plasma (PRP), and others applied for burn wound healing are addressed in this paper.

Keywords

Burn injury · Wound healing · Advanced techniques · Negative pressure wound therapy · Platelet-rich plasma

19.1 Introduction

Burn injury is the damage of the structure and function of the skin caused by heat/thermal, flame, electricity, chemicals, and radiation, followed by the pathophysiological changes and serious complications such as inhalation injury, hypovolemic shock, infection, visceral complication, organ failure, and so on [1]. According to the latest report of the World Health Organization (updated August 2017), about 180,000 deaths are caused by burns every year. The vast majority of burn accidents occur in low- and middle-income countries, and almost two thirds occur in the African and Southeast Asia regions such as India, Bangladesh, Colombia, Egypt,

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and Pakistan. The elder people and young children meet a relative high mortality rate [2]. In the developed countries, burn death rates have been decreasing; the rate of child deaths from burns is over seven times higher in low- and middle-income countries. Females have slightly higher rates of death from burns compared to males according to the most recent data.

Severe burn injuries cannot only lead to local damages but also systemic pathological changes and even death. Due to the disfigurement and disability, the burn victims suffer stigma and rejection from the surround people resulting in severe mental and emotional distresses. Moreover, the long hospitalization, repeated care for deformities, lost wages, commitment of family, and society resources contributed to the socioeconomic burden.

Fortunately, the survival rates of burns have increased significantly over the last century due to the advancements of burn care and therapy [3]. With the further technological improvements such as accurate diagnosis of burn wounds, early surgical intervention, antimicrobial therapy, critical care support, and wound care, the burn patients receive the shorter length of hospital stay, decreased operation times, and better quality of life. Comprehensive therapy in burns begins with initial accurate evaluation of the wounds, followed by the resuscitation, timely excision, grafting, and closure. Therefore, the advanced techniques in burn wound repair are involved in a diverse breadth of skills, including new method of diagnosis, advanced methods to prevent infection, new skills to close the wounds, and photoelectric treatment to improve wound healing and tissue regeneration.

19.2 Early Precise Diagnosis of Burn Wounds

Burn area and depth are the main aspects for the burn diagnosis. The depth of burn injury is the most important factor to choose the treatment method and affect the quality of wound healing. At present, even in the developed countries such as the United State, the burn depth is still primarily based on a visual assessment by the clinical doctors with their experiences. However the accuracy diagnosis of burn depth by this routine method is only 60–75%, which is far from the precise treatment [4]. Therefore, the advanced equipments and technologies such as wound temperature measurement, ultrasonic imaging, tissue staining, video imaging, and laser Doppler imaging (LDI) are applied to assess the depth of burn injury [5]. These technologies can provide more information regarding necrotic, degenerated skin tissue objectively, which is helpful to determine the burn depth precisely. In 1975, the LDI was firstly applied in the detection of blood flow. And it was used to evaluate burn depth as an objective and effective measurement since 1984. At present, LDI has been reported to be an accurate diagnostic tool with high sensitivity and specificity in the assessment of the superficial II degree, deep superficial degree, and full-thickness burn wounds. It was said that the accuracy rate of LDI to assay the depth of burn wound without infection was 99%, and it can predict the outcome of different depth burn wound as precise as 98.4% [6]. Lotter found that LDI combined with a tissue spectrophotometer can discriminate various burn depths of injury in

the minipig model by analyzing the blood flow and total and oxygenated hemoglobin [7]. Ida developed a real-time (8–30 fps) photoacoustic (PA) imaging system with a linear array transducer for burn depth assessment, which could distinguish and image the devitalized tissue in burn wound from the normal skin [8]. Laser speckle contrast analysis (LASCA) is another optical technique to tell the burn tissue from the normal tissue by visualized the relative blood perfusion [9]. Moreover, some new noncontact technologies such as forward-looking infrared (FLIR) image, active dynamic thermography (ADT), optical coherence tomography (OCT), and pulse speckle imaging (PSI) make it possible to visualize the burned tissue more sensitive and more specific [10, 11]. Our team used the near-infrared spectroscopy (NIFS) to detect the burn depth in order to make an accurate diagnosis on burn depth, which will be the first equipment of the objective diagnosis on the burn depth in China.

19.3 Proper Treatment of Burn Wounds

The skin plays a pivotal role to protect the body from harmful external conditions, maintain the body fluid homeostasis, thermoregulation, and regulate many metabolic processes. The skin is also the body's largest and most active immune organ to against microbial infection as the first line of defense. Patients suffered with burn injury, especial the severe burn injury, should be cared immediately, and the mechanical barrier should be reconstructed shortly. Hence to achieve faster reepithelialization or closure of burn wounds, more and more researchers and surgeons have been exploring new strategies and techniques to decrease the degree or depth of burn injury, which is the main factor to determine the outcome or consequence of wound healing and the victims' selves. The strategies include modified and standardized cooling therapy, rational treatment and surgical intervention of the burn wound, application of new functional wound dressings and drugs, photoelectric therapy, and others.

19.3.1 Modified and Standardized Cooling Therapy

Cooling the burn area is one of the most practical and effective method to reduce burn injuries. It was found that this method was recommended by Dr. Galen as a first aid since AD150. The cooling therapy has the beneficial effects to rapidly reduce tissue temperature and thus limit the devastation from a burn. In addition, cooling the burn area can decrease pain, lower the infection rate, promise a shorter recovery period, and lessen the need for grafting. It is found that the injury of the skin will be continued as long as the temperature is above 44 °C [12]. The effect of immediate cooling after burn is better than the delayed cooling. The best way for cooling therapy is to rinse the suffered areas with clean water or tap water as early as you can. The recommendation time for cooling therapy is as long as 30 min to 1 h after burn injury. It should be much more effective for the chemical burn injury to

start the cooling as soon as possible and prolong the therapy time to 1 h and more [13]. Dr. Baldwin found that flushing a wound with water is marginally more effective than immersing the wound into a cold water bath and more effective than cooling the burn by natural convection of air [13]. Dr. Davies concluded that rapid reduction of skin temperature could decrease the extent of injury by cooling therapy, which can significantly improve wound healing and reduce surgical intervention [14]. Dr. Rizzo indicated that immediate hypothermia decreased burn depth progression at 6 h post injury, and this protective effect was sustained for at least 24 h. The burn depth was 23% lower than that in the control group at 24 h. Even cooling therapy delayed at 2 h postburn, the burn depth was still decreased 18% lower. It was found that the temperature of burn areas reduced to below 40 °C within 2 min that results in the good outcome; however, it was still effective to cool the burn areas 30 min later post injury [15]. It is clear that clean water or tap water at room temperature is the best choice for cooling therapy; it is practical and effective.

19.3.2 Surgical Intervention

Early excision of the burn eschar has been one of the most significant advances in modern burn care [16]. As early as in 1920s, people recognized the importance to remove early the necrotic and denatured tissues in the burn wound treatment, and gradually this method became the main treatment way of deep burn wounds [17]. Dr. Keshavarzi reported that ultra-early (48–72 h) excision and grafting in burn patients less than 60% TBSA were associated with higher graft success rate, shorter length of hospital stay, lower infection rate, and lower mortality compared with these operated in 7–10 days [18]. According to the implementation time, the early escharectomy or tangential excision of deep burn wounds can be divided into three types, i.e., primary stage, prompt stage, and early stage. The primary stage means the surgery carried out within 24 h after burn injury. The prompt stage refers to 24–72 h after burn, while the early stage refers to 3–5 days. In 1950s, early extensive escharectomy emerged and introduced by Dr. Braw as a new method to treat the major burn patients. In 1979, professor Sheng in No.304 Hospital Beijing of China began to do extensive escharectomy within 48 h after burn injury. Dr. Huang in Third Military Medical University of China in 1998 proposed extensive totally escharectomy on severe burn patients at within or around 24 h after burn injury, which achieved good outcome not only in wound healing but also in decreasing incidences of different complications and mortality. This method is still used in clinical practice in some burn centers. It is necessary to evaluate carefully the general status, especially the circulation stability, before this kind of operation. However, in some underdeveloped area such as in sub-Saharan Africa, it was found that early escharectomy or tangential excision and grafting with 5 days after burn injury resulted in a significant higher mortality compared with that operated later [19].

Debridement is a fundamental principle in the management of burn wounds, which can remove the necrotic tissues, decrease the infection rate, and promote wound healing. There are many advanced methods and equipments for the

debridement of burn wound, i.e., water jet, ultrasonic, biological, chemical, and others. Enzymatic debridement (ED) belongs to chemical non-operative debridement. It was first used in burn treatment since the Second World War, which can delete the necrotic and degenerated tissue completely, while reserve the vital tissue and appearance maximally. Dr. Cordts reported that ED reduced the possibility of surgical escharectomy and skin grafting and minimized complications. Moreover, ED could improve quality of wound healing and reduce contractures and aesthetic disadvantages significantly [20]. Compared with the traditional surgical debridement of severely burned hands, Schulz found that enzymatic debridement with bromelain markedly reduced the waiting time for debridement and the time consuming of manipulation [21]. Versajet™ Hydrosurgery System (Smith & Nephew, Hull, UK) is a novel water-jet dissection device designed to surgically debride burn wounds, which can reduce collateral damage, and subsequently achieve overall better outcomes than conventional debridement techniques [22]. After debridement, the quality of the wound bed was ideal for accelerating endogenous burn wound healing. Dr. Klein thought this device even had a benefit in small areas for excision of burns of the eyelids, digits, and web spaces, providing a relatively facile method for excision of challenging aesthetic and functional areas [23].

Transplantation of healthy human skin to damaged area is still the gold standard in the treatment of deep or full-thickness burn wounds. However, challenges arise when large areas are affected and donor sites are scarce. Therefore many skin graft expansion techniques have been developed to reduce the donor size, including meshing of the graft (maximum expansion ratio of 1:9), modified meek technique (expansion ratio of 1:9), epidermal blister grafting (expansion ratio of 1:1), several techniques of micro-grafting such as epidermal CelluTome micro-grafting (expansion ratio: 1:6) or Xpansion micro-grafting (maximum expansion ratio: 1:100), cultured epithelial sheets (expansion rate 1:1000), and uncultured cell suspensions (maximum expansion rate 1:100) [24–26].

In China, microskin graft has been becoming a routine method to repair large size burn wound and rescues lots of lives of severe burn patients. Only a small piece of skin is taken in this method, and then the skin is cut with scissors into very small particles. After all the necrosis and degenerated tissues are escharectomied or excised away, the auto-microskin is seeded onto the prepared wounds evenly. After that, biomaterial dressings such as alloskin or porcine skin are always used to cover the surgical burn area, which can provide a suitable microenvironment for the survival, growth, and expansion of the microskin seeded on the wound. The ratio of donor and grafted area is usually around 1:8–10. After 3–4 weeks, the burn wound heals with the growth and expansion of the little skin particles under the biodressing.

19.3.3 Wound Dressing

Application of wound dressings is the principle therapeutic intervention for the burn patients. The average commercial value of wound dressings is estimated to be

around 4–9 billion dollars, and it will grow by 4–7% every year until 2021 [27]. Traditional wound dressing (TWD) was designed to just cover the wound and played no active role in wound healing and skin regeneration. But the advanced active wound dressings aim to enhance the natural healing process and work to counter many aspects that plague poorly healing wounds, including excessive inflammation, ischemia, scarring, and wound infection, which enhanced healing and bridge the gaps in the healing processes that prevent wound from healing. In burn wounds, most of the wound dressings are the hydrophilic materials such as hydrogels, collagen, and peptides, which can prevent water loss. Dr. Mohamad reported that bacterial cellulose/acrylic acid (BC/AA) hydrogels promoted faster wound healing, enhanced epithelialization, and accelerated fibroblast proliferation with nontoxic [28]. Dr. Strong described their results with the application of fetal bovine collagen (FBC) matrix on full-thickness burn wound; he found that the wound healed without the need of subsequent skin grafting [29]. Dr. Yergoz found that the heparin-mimetic peptide nanofiber gels increased the healing of burn wounds [30]. Dhall developed an alginate sponge dressing (ASD) containing insulin encapsulated in PLGA (poly(D,L-lactic-co-glycolic acid)) microparticles which could accelerate burn healing and stimulates a more regenerative, less scarring healing [31]. The collagen, alginate, and hydrogel are natural polymers, which have a structural elements common with normal ECM, thus they offer good cell attachment and preserve cell viability. In addition, there are synthetic materials such as nanofibers, PU, and nanoparticle used as the burn wound dressings [32]. The nanoparticles such as Ag, ZnO, nanoceria, TiO_2, Cu, Fe_3O_4, Al_2O_3, and SiO_2 have antibacterial properties to against infection. Some nanoparticles have been used as a carrier for other molecules such as alpha lipoic acid and epigallocatechin gallate (EGCG) that act to regulate inflammation and angiogenesis. Gold nanoparticles control the production of ROS and show antioxidant properties [33]. Dressings with nano-platelet graphene oxide (GO) resulted in better vascularization, reepithelization (due to controlled moisture), and improved collagen regeneration compared with the non-GO counterpart [34].

19.3.4 Negative Pressure Wound Therapy

Negative pressure wound therapy (NPWT), used in the treatment of acute wound for almost 20 years, can help manage infected wounds after applied along with appropriate debridement and antibiotic therapy. NPWT not only removes fluid and reduces edema but also promotes perfusion around the wounds [35]. NPWT can help to prevent postoperative infection and promote granulation tissue formation to prepare the wound bed for subsequent skin graft. Meanwhile NPWT is used in burn care as a skin graft bolster or a dressing for skin graft donor sites [36]. In 2004, the first application of NPWT in the acute management of burn wounds was studied in hand burns by Kamolz, which got the conclusion that the burn patients with partial-thickness or mixed-thickness burn may benefit from

the application of subatmospheric pressure by reducing edema formation and increasing perfusion [37]. Fischer used extra-large NPWT to treat ≥15% TBSA burn patients and found the extra-large NPWT appeared to improve graft take and to decrease risk of infection, pain, and anxiety associated with wound care [38]. Low also suggested to use NPWT to enhanced total body wrap to promote healing in burns [39]. NPWT improved rate of revascularization when used over dermal substitutes and increased rate of reepithelialization when used over skin graft donor sites [40]. However there was not enough evidence from randomized controlled trials (RCTs) available to get any conclusions of using NPWT for treatment of partial-thickness burn wounds [41].

19.3.5 Phototherapy

Phototherapy on burn wound can accelerate epithelialization and wound healing, reduce pain, and hasten the resolution of inflammation. It includes the nonthermal levels of red or near-infrared (NIR) light, light-emitting diode (LED), UV irradiation, blue light, and others for phototherapy on burn wound. The red/NIR light can penetrate into tissue to stimulate mitochondrial respiration and trigger transcription of new gene products. UV light could be used as a viable approach for infection therapy in burn patients [42]. Blue light has antimicrobial effects, especially for treatment of infected burn wounds. Dai demonstrated that blue light (451 nm) plays approximately 35-fold higher antibacterial of *P. aeruginosa* than damage on keratinocytes [43]. Mester demonstrated that laser at low dosage increased hair growth and promoted excisional wound healing [44]. This observation was termed "laser biostimulation." Since then, the specialized field of phototherapy that utilizes low-dose light for clinical therapy was reborn. Dr. Silveira compared the differences of low power laser and LED on burn wound; they found both of these two lights could reduce the inflammatory response and decrease oxidative stress parameters, which resulted in high speed and good quality of wound [45]. The results of Fekrazad's study showed that using therapeutic lasers with green, blue, red, and infrared light could accelerate healing process. Moreover it was found that red and infrared lights had more obvious effects, especially during the acute phase [46].

19.3.6 Ultrasound Therapy

Ultrasound, traveling through tissue as acoustic wave, has been indicated to have advantageous effects on wound healing in burns. Dr. Fantinati investigated the effect of ultrasound on third-degree burn wounds; they found that low-intensity ultrasound could reduce the progressive necrosis of wound, increase the granulation tissue formation, as well as accelerate wound closure and reepithelization [47]. This result indicated that ultrasound should be beneficial in the inflammatory and proliferative

phases of the wound healing process. It is possible to think that the ultrasound could induce undesirable angiogenesis as well as inflammation in the remodeling phase. Therefore, the ultrasound should be only used during the early phase of wound healing. However, Dr. Mesquita reported that low-intensity therapeutic ultrasound improved body weight and granulation tissue formation, but did not influence the wound healing of burns in rats [48]. There should be much more research to explore the exact effect of ultrasound on burn wound healing.

19.3.7 Platelet Rich Plasma (PRP)

The topical administration of autologous PRP as a new technique of biological accelerator on the wound healing has been safely applied since the 1990s [49]. Platelet releases a vast array of granular components and biological mediators, such as growth factors, immune regulators, antimicrobial peptides, and others. It was proven that PRP can stimulate angiogenesis and promote vascular growth and fibroblast proliferation, which enhance wound healing in both soft and hard tissue. Prof. Venter found PRP could accelerate the healing process in deep second-degree burns not only in normal rats but also in diabetic rats. And it is clear that PRP did not have any effect on third-degree burns of rats [50]. Maciel's study showed PRP could provide antibacterial activity and induce fibrosis in deep second-degree burns of horses [51]. However Marck reported the addition of PRP did not result in improved graft take and epithelialization nor better scar quality [52].

19.4 Conclusion

Skin is the body's largest organ to against the damage from external environment as the first barrier. The skin barrier destroyed by burn injury leads to loss of water, electrolytes, energy and nutritional components, secondary infection, and even death. However treatment of severe burns poses a complex problem in medical care. The key point is how to heal the burn wound and reconstruct the mechanical and biological barrier as soon as possible. Moreover, the best results are to repair the burn wound quickly without any scar formation and contracture. The ultimate goal is to regenerate skin tissue and barrier as same as the original structure. Firstly, we should make precise diagnosis of burn wounds objectively but not only by our eyes, especially of the depth of burns with advanced devices. Secondly, the exposed area of the burn wound should be covered with a wound dressing, followed by excision and graft surgery. Meanwhile the methods with NPWT, photo/ultrasound therapy, PRP, and others are better choices to be applied on the burn wound. Of course, more advanced techniques will continue to be explored in the diagnosis and treatment of burn wound. We hope that the burn wound may repair shortly without scar and without huge financial burden in the not-so-far future.

References

1. Oryan A, Alemzadeh E, Moshiri A. Burn wound healing: present concepts, treatment strategies and future directions. J Wound Care. 2017;26:5–19.
2. Jackson PC, Hardwicke J, Bamford A, Nightingale P, Wilson Y, Papini R, et al. Revised estimates of mortality from the Birmingham Burn Centre, 2001–2010: a continuing analysis over 65 years. Ann Surg. 2014;259:979–84.
3. Osler T, Glance LG, Hosmer DW. Simplified estimates of the probability of death after burn injuries: extending and updating the baux score. J Trauma. 2010;68:690–7.
4. Resch TR, Drake RM, Helmer SD, Jost GD, Osland JS. Estimation of burn depth at burn centers in the United States: a survey. J Burn Care Res. 2014;35:491–7.
5. Paul DW, Ghassemi P, Ramella-Roman JC, Prindeze NJ, Moffatt LT, Alkhalil A, et al. Noninvasive imaging technologies for cutaneous wound assessment: a review. Wound Repair Regen. 2015;23:149–62.
6. Shin JY, Yi HS. Diagnostic accuracy of laser Doppler imaging in burn depth assessment: systematic review and meta-analysis. Burns. 2016;42:1369–76.
7. Lotter O, Held M, Schiefer J, Werner O, Medved F, Schaller HE, et al. Utilization of laser Doppler flowmetry and tissue spectrophotometry for burn depth assessment using a miniature swine model. Wound Repair Regen. 2015;23:132–6.
8. Ida T, Kawaguchi Y, Kawauchi S, Iwaya K, Tsuda H, Saitoh D, et al. Real-time photoacoustic imaging system for burn diagnosis. J Biomed Opt. 2014;19:086013.
9. Ragol S, Remer I, Shoham Y, Hazan S, Willenz U, Sinelnikov I, et al. Static laser speckle contrast analysis for noninvasive burn diagnosis using a camera-phone imager. J Biomed Opt. 2015;20:86009.
10. Renkielska A, Kaczmarek M, Nowakowski A, Grudzinski J, Czapiewski P, Krajewski A, et al. Active dynamic infrared thermal imaging in burn depth evaluation. J Burn Care Res. 2014;35:e294–303.
11. Ganapathy P, Tamminedi T, Qin Y, Nanney L, Cardwell N, Pollins A, et al. Dual-imaging system for burn depth diagnosis. Burns. 2014;40:67–81.
12. Wright EH, Harris AL, Furniss D. Cooling of burns: mechanisms and models. Burns. 2015;41:882–9.
13. Baldwin A, Xu J, Attinger D. How to cool a burn: a heat transfer point of view. J Burn Care Res. 2012;33:176–87.
14. Davies JW. Prompt cooling of burned areas: a review of benefits and the effector mechanisms. Burns Incl Therm Inj. 1982;9:1–6.
15. Rizzo JA, Burgess P, Cartie RJ, Prasad BM. Moderate systemic hypothermia decreases burn depth progression. Burns. 2013;39:436–44.
16. Mosier MJ, Gibran NS. Surgical excision of the burn wound. Clin Plast Surg. 2009;36:617–25.
17. Gacto-Sanchez P. Surgical treatment and management of the severely burn patient: review and update. Med Intensiva. 2017;41:356–64.
18. Keshavarzi A, Ayaz M, Dehghankhalili M. Ultra-early versus early excision and grafting for thermal burns up to 60% total body surface area; a historical cohort study. Bull Emerg Trauma. 2016;4:197–201.
19. Gallaher JR, Mjuweni S, Shah M, Cairns BA, Charles AG. Timing of early excision and grafting following burn in sub-Saharan Africa. Burns. 2015;41:1353–9.
20. Cordts T, Horter J, Vogelpohl J, Kremer T, Kneser U, Hernekamp JF. Enzymatic debridement for the treatment of severely burned upper extremities - early single center experiences. BMC Dermatol. 2016;16:8.
21. Schulz A, Shoham Y, Rosenberg L, Rothermund I, Perbix W, Christian Fuchs P, et al. Enzymatic versus traditional surgical debridement of severely burned hands: a comparison of selectivity, efficacy, healing time, and three-month scar quality. J Burn Care Res. 2017;38:e745–e55.
22. Kakagia DD, Karadimas EJ. The efficacy of versajet hydrosurgery system in burn surgery. A systematic review. J Burn Care Res. 2018;39(2):188–200.

23. Klein MB, Hunter S, Heimbach DM, Engrav LH, Honari S, Gallery E, et al. The Versajet water dissector: a new tool for tangential excision. J Burn Care Rehabil. 2005;26:483–7.
24. Kadam D. Novel expansion techniques for skin grafts. Indian J Plast Surg. 2016;49:5–15.
25. Singh M, Nuutila K, Kruse C, Robson MC, Caterson E, Eriksson E. Challenging the conventional therapy: emerging skin graft techniques for wound healing. Plast Reconstr Surg. 2015;136:524e–30e.
26. Ter Horst B, Chouhan G, Moiemen NS, Grover LM. Advances in keratinocyte delivery in burn wound care. Adv Drug Deliv Rev. 2018;123:18–32.
27. Das S, Baker AB. Biomaterials and nanotherapeutics for enhancing skin wound healing. Front Bioeng Biotechnol. 2016;4:82.
28. Mohamad N, Mohd Amin MC, Pandey M, Ahmad N, Rajab NF. Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: accelerated burn wound healing in an animal model. Carbohydr Polym. 2014;114:312–20.
29. Strong AL, Bennett DK, Spreen EB, Adhvaryu DV, Littleton JC, Mencer EJ. Fetal bovine collagen matrix in the treatment of a full thickness burn wound: a case report with long-term follow-up. J Burn Care Res. 2016;37:e292–7.
30. Yergoz F, Hastar N, Cimenci CE, Ozkan AD, Tekinay T, Guler MO, et al. Heparin mimetic peptide nanofiber gel promotes regeneration of full thickness burn injury. Biomaterials. 2017;134:117–27.
31. Dhall S, Silva JP, Liu Y, Hrynyk M, Garcia M, Chan A, et al. Release of insulin from PLGA-alginate dressing stimulates regenerative healing of burn wounds in rats. Clin Sci (Lond). 2015;129:1115–29.
32. Mofazzal Jahromi MA, Sahandi Zangabad P, Moosavi Basri SM, Sahandi Zangabad K, Ghamarypour A, Aref AR, et al. Nanomedicine and advanced technologies for burns: preventing infection and facilitating wound healing. Adv Drug Deliv Rev. 2018;123:33–64.
33. Zarrintaj P, Moghaddam AS, Manouchehri S, Atoufi Z, Amiri A, Amirkhani MA, et al. Can regenerative medicine and nanotechnology combine to heal wounds? The search for the ideal wound dressing. Nanomedicine (Lond). 2017;12(19):2403–22.
34. Shams E, Yeganeh H, Naderi-Manesh H, Gharibi R, Mohammad Hassan Z. Polyurethane/siloxane membranes containing graphene oxide nanoplatelets as antimicrobial wound dressings: in vitro and in vivo evaluations. J Mater Sci Mater Med. 2017;28:75.
35. Teng SC. Use of negative pressure wound therapy in burn patients. Int Wound J. 2016;13(Suppl 3):15–8.
36. Kantak NA, Mistry R, Varon DE, Halvorson EG. Negative pressure wound therapy for burns. Clin Plast Surg. 2017;44:671–7.
37. Kamolz LP, Andel H, Haslik W, Winter W, Meissl G, Frey M. Use of subatmospheric pressure therapy to prevent burn wound progression in human: first experiences. Burns. 2004;30:253–8.
38. Fischer S, Wall J, Pomahac B, Riviello R, Halvorson EG. Extra-large negative pressure wound therapy dressings for burns - initial experience with technique, fluid management, and outcomes. Burns. 2016;42:457–65.
39. Low OW, Chong SJ, Tan BK. The enhanced total body wrap--the new frontier in dressing care for burns. Burns. 2013;39:1420–2.
40. Kantak NA, Mistry R, Halvorson EG. A review of negative-pressure wound therapy in the management of burn wounds. Burns. 2016;42:1623–33.
41. Dumville JC, Munson C, Christie J. Negative pressure wound therapy for partial-thickness burns. Cochrane Database Syst Rev. 2014;12:Cd006215.
42. Aleem NA, Aslam M, Zahid MF, Rahman AJ, Rehman FU. Treatment of burn wound infection using ultraviolet light: a case report. J Am Coll Clin Wound Spec. 2013;5:19–22.
43. Dai T, Gupta A, Huang YY, Yin R, Murray CK, Vrahas MS, et al. Blue light rescues mice from potentially fatal Pseudomonas aeruginosa burn infection: efficacy, safety, and mechanism of action. Antimicrob Agents Chemother. 2013;57:1238–45.
44. Mester E, Szende B, Gartner P. The effect of laser beams on the growth of hair in mice. Radiobiol Radiother. 1968;9:621–6.

45. Silveira PC, Ferreira KB, da Rocha FR, Pieri BL, Pedroso GS, De Souza CT, et al. Effect of low-power laser (LPL) and light-emitting diode (LED) on inflammatory response in burn wound healing. Inflammation. 2016;39:1395–404.
46. Fekrazad R, Nikkerdar A, Joharchi K, Kalhori KA, Mashhadi Abbas F, Salimi Vahid F. Evaluation of therapeutic laser influences on the healing of third-degree burns in rats according to different wavelengths. J Cosmet Laser Ther. 2017;19:232–6.
47. Fantinati MS, Mendonca DE, Fantinati AM, Santos BF, Reis JC, Afonso CL, et al. Low intensity ultrasound therapy induces angiogenesis and persistent inflammation in the chronic phase of the healing process of third degree burn wounds experimentally induced in diabetic and non-diabetic rats. Acta Cir Bras. 2016;31:463–71.
48. Mesquita RL, Silva PI, Melo e Silva SH, Oliveira KO, Fontes-Pereira AJ, Freitas JJ, et al. Effect of low-intensity therapeutic ultrasound on wound healing in rats subjected to third-degree burns. Acta Cir Bras. 2016;31:36–43.
49. Martinez-Zapata MJ, Marti-Carvajal A, Sola I, Bolibar I, Angel Exposito J, Rodriguez L, et al. Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: a systematic review. Transfusion. 2009;49:44–56.
50. Venter NG, Marques RG, Santos JS, Monte-Alto-Costa A. Use of platelet-rich plasma in deep second- and third-degree burns. Burns. 2016;42:807–14.
51. Maciel FB, DeRossi R, Modolo TJ, Pagliosa RC, Leal CR, Delben AA. Scanning electron microscopy and microbiological evaluation of equine burn wound repair after platelet-rich plasma gel treatment. Burns. 2012;38:1058–65.
52. Marck RE, Gardien KL, Stekelenburg CM, Vehmeijer M, Baas D, Tuinebreijer WE, et al. The application of platelet-rich plasma in the treatment of deep dermal burns: a randomized, double-blind, intra-patient controlled study. Wound Repair Regen. 2016;24:712–20.

20 Plasticity of Epidermal Stem Cells: The Future of Stem Cell-Based Therapeutics to Improve Cutaneous Wound Healing

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Abstract

The purpose of wound healing is to repair the skin to prevent infection and to restore tissue integrity and function. Unfortunately, in adults, this process is geared toward faster rates of healing, to prevent infection, which ultimately leads to a compromise in the quality of healing. This compromise results in scarring, where the architecture of the skin is distinct from the original tissue, significantly affecting function and overall quality of life. The ideal for future treatments is to increase the rates of healing while improving the quality of healing resulting in more of a regenerative process rather than a repair-orientated one.

The use of cellular therapy in the treatment of cutaneous wounds is currently an active area of investigation. Multipotent adult stem cells are an attractive choice for cell therapy because they have a large proliferative potential, the ability to differentiate into different cell types and produce a variety of cytokines and growth factors important to wound healing. As the biggest organ in the body, skin tissues represent a larger reservoir for adult stem cells. Recent studies further report that adult skin tissues contain cell populations with pluripotent characteristics. Multipotent stem cells from hair follicle and non-follicular skin, both in epidermal and dermal tissues, are found to have the differentiation capacity to generate multiple cell lineages. Specifically, it has been evidenced that keratinocytes in the skin may possess a transcriptional profile that is more amenable to reprogramming, and the fate of these cells can change in response to surrounding microenvironment. Given its easy accessibility, stem cells in the skin will not only provide an experimental model for skin biology but also may have extensive therapeutic implications in the replacement of the skin and may serve as an alternative source of stem cells for several other organs outside of the skin. The in situ activation and mobilization of stem cell populations in the skin is an ideal way to renew and repair the epidermis and dermis, even appendages.

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20.1 Introduction

The skin is a powerful barrier to defend our body against a variety of environmental insults and helps to maintain fluid balance within the body. The skin also performs a wide range of functions, including sensation, heat regulation, synthesis, absorption, as well as control of evaporation. Skin diseases, such as deficient wound healing, can lead to loss of function and decreased quality of life and are a significant cause of morbidity. In developed countries, it has been reported that about 1–2% of the population will experience a chronic wound during their lifetime [1, 2]. In the United States, an estimated 18% of diabetic patients over the age of 65 suffer from nonhealing foot ulcers [3]. Worldwide, a lower limb or part of a lower limb is lost every 30 s as a result of diabetic wound infection. Therefore, chronic wounds represent a major challenge for the clinician and wound care specialist. Understanding and addressing the biological mechanisms of cutaneous wound healing will offer new hope for clinical treatment, resulting in improved patient quality of life and reduced healthcare costs.

Cutaneous wound healing is a dynamic, well-organized, and complex process, which consists of an inflammatory phase, new tissue formation, and remodeling phase [4]. When injuries to the skin occur, tissue injury disrupts vascular vessels and the release of growth factors, cytokines, and components of ECM initiates inflammatory response. Then, the proliferative phase of tissue repair is achieved by the migration and hyperproliferation of dermal and epidermal cells within the wound bed. The new tissue that forms at the wound site is called granulation tissue because of the granular appearance composed of numerous capillaries, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional extracellular matrix (ECM). The formation of granulation tissue into the wound bed allows the reepithelialization to take place. The keratinocytes at the wound site undergo morphologic alterations, changing from sedentary cells to migratory cells. The phenotype of the migratory cells relies on an EMT-like process to impart migratory ability to the epithelial cells localized to the wound edges, which allowed them to shed their cell-cell adhesions, lose their apical-basal polarity, dissolve the basement membrane, and reorganize their cytoskeletal structure to generate cytoplasmic extensions such as lamellipodia. Studies have shown that keratinocytes at the nonhealing edges of chronic wounds are different both phenotypically and biologically from keratinocytes comprising intact epidermis or the edge of acute wounds [5, 6]. Several studies have also examined vimentin [7] and Slug [8, 9] expressions in skin injury models to provide evidence for the acquisition of mesenchymal-like phenotype during cutaneous wound healing. During the reepithelialization, keratinocytes at the wound edge conduct migration, proliferation, and

differentiation and reconstitute the epidermal integrity over the granulation tissue ultimately. The tissue remodeling phase is characterized by matrix remodeling and declined cellularity. During this phase the collagen fibers reorganize and mature to gain tensile strength in the end. Generally speaking, an acute wound with traumatic or surgical origin could be healed without significant interventions, whereas repeated infection of damaged tissues is the main reason for wound chronicity, morbidity, and mortality. Although chronic or acute wounds involved multiple pathophysiological mechanisms, both processes required the coordinated and temporal orchestration of a variety of cells and factors which develop in a precisely controlled course to regenerate damaged structures with the correct size and shape. Particularly, it is believed that endogenous stem cells play an important role in the well-coordinated cell-signaling cascades of wound healing. The skin, the largest organ in the body, consists of a keratinized stratified epidermis and a thick underlying layer of collagen-rich dermal connective tissue. The skin epidermis and its appendages (hair follicles, sebaceous glands, and sweat glands) harbor spatially distinct stem cell niches, which represent a larger reservoir for adult stem cells (including epidermal, mesenchymal, hematopoietic, and neural stem cells). These cells not only play a pivotal role in the functional repair of the skin itself but will also offer a potential source of adult stem cells for the cell-based therapy of injuries and diseases throughout the body. Emerging evidence now indicated that lineage-specific epidermal stem cells, irrespective of their ancestry, have the potential to contribute to essentially all the pilosebaceous unit (PSU) compartments when provided with an appropriate microenvironment.

In this chapter, we will present an overview of recent work in research into cutaneous repair and regeneration involving stem cells from the epidermis, dermis, and bone marrow, concentrating in particular on the latest developments in the occurrence, plasticity, and potential uses of skin stem cells. Such research can provide new insights into the molecular mechanisms underlying the cellular conversions between different cell lineages and a platform for producing patient-specific cell therapies that avoid immune rejection and tumorigenesis.

20.2 Epidermal Development: An Overview

Skin morphogenesis occurs following a continuous series of cell-cell interactions which can be subdivided into three main stages: the formation of a homogeneous embryonic skin, composed of an epidermis overlying a dense dermis, the organization of these primary homogeneous fields into heterogeneous ones by the appearance of cutaneous appendage primordial, and the final step, cutaneous appendage organogenesis itself. However, before skin morphogenesis, various cellular interactions arise from the embryonic ectoderm, which specify first the formation of dermal progenitors [10]. It should be noted that at the beginning of embryogenesis, the embryo surface emerges as a single layer of neuroectoderm, which will ultimately give rise to two distinct lineages: these are the ectoderm proper, which is the source of the epidermis and the corneal, nasal, vaginal, and part of the oral epithelia, and

the neuroderm, which gives rise to the cells of the central nervous system and neural crest cells. At the crossroads of this decision is the Wnt pathway, which is associated first with the formation of the dorsal dermis and blocks the ability of early ectodermal progenitor cells to respond to fibroblast growth factors (FGFs), a proposed neural inductor [11], allowing them to respond to BMP signaling and become fated to develop into the epidermis. Conversely, in the absence of a Wnt signal, the ectoderm is able to receive and translate activating cues by FGFs, downregulate BMP signaling, and progress toward neurogenesis [12]. As a result, the embryonic epidermis that results from this process consists of a single layer of homogeneous progenitor cells—epidermal stem cells. Epidermal stem cells reside in the basal layer of the epidermis, which can undergo self-renewal, thereby giving rise to another stem cell, or can divide to give rise to transit amplifying cells (TA cells). Subsequently, TA cells cease cell cycle activity, detach from the basement membrane, and begin the complex process of terminal differentiation. As cells differentiate, they move up to form the different layers of the epidermis (spinous, granular, and cornified layers) and are eventually lost from the skin surface [13].

20.3 Hair Follicle Morphogenesis: Epithelial-Mesenchymal Interactions

In vertebrates, the formation of the hair follicle and its cyclical growth, quiescence, and regeneration depend on reciprocal signaling between its epidermal and dermal components. Pioneering studies on mesenchymal-epithelial tissue recombination in chicks and mice revealed that early cues from the mesenchyme derived from the dermomyotome can instruct the overlying ectoderm to commit to forming a hair placode, which appears as small epidermal invaginations into the underlying dermis [14]. As the epithelium thickens, hair placode or germ sends messages back to the underlying mesenchyme, stimulating their condensation to form the future dermal papilla [15]. A third signal from the condensed mesoderm then induces proliferation of the overlying ectodermal placode allowing it to grow downward eventually surrounding the mesenchymal condensation. The latter forms the dermal papilla, the permanent mesenchymal component of the hair follicle. As development proceeds, the epithelial cells differentiate under the influence of unidentified morphogens, producing first the outer root sheath (ORS), which is contiguous with the basal layer of the epidermis, and the inner root sheath (IRS), which molds and guides the shaft in its passage outward as a unit. The epithelial cells forming a cloak surrounding the dermal papilla are called matrix cells, which proliferate transiently. As matrix cells withdraw from the cell cycle, they differentiate into upwardly moving cells. At the center, matrix cells differentiate into precortical cells, which subsequently give rise to three concentric cylinders constituting the medulla, cortex, and cuticle of the hair shaft at the center. Near the skin surface, the IRS degenerates, freeing the hair shaft to push outward as matrix cells proliferate and differentiate at the base. Once established, follicles proceed through cycles of active growth (anagen), regression (catagen), and rest (telogen).

Therefore, the specialized mesenchymal cells, or called the dermal papilla (DP), are essential to hair follicle morphogenesis. An excellent marker of mouse papilla cells is alkaline phosphatase, which, in contrast to previous studies, has been found to be expressed throughout the cycle [16]. However, it is of interest that the blastema of regenerating newt limb is also rich in alkaline phosphatase expression with respect to the regeneration concept [17]. Another typical feature of papilla cells in vivo and in vitro is that they have a tendency to aggregate. In that regard, it is of interest that throughout the cycle, papilla cells can express neural cell adhesion molecule (NCAM) [18], a molecule mediating cell-to-cell and cell-to-matrix adhesion. Simultaneously, the papilla-matrix basement membrane zone (BMZ) becomes fenestrated in culture and after retinoid treatment during follicle morphogenesis, allowing processes from DP cells to contact epithelial hair matrix cells, an important relationship that appears to play a role in reciprocal epithelial-mesenchymal cross talk [19]. Taken together, the dermal papilla is an inductive structure that sends and receives signals in the regulation of hair follicle formation. When DPs are dissected and combined with hair follicle fragments containing bulge stem cells, they can reconstitute a viable hair follicle when grafted to a nude mouse host [20]. Especially, cells or factors present in fresh, but not cultured, dermal cells are essential for supporting hair growth from budding follicles, whereas hair follicle buds grafted alone or with cultured dermal cells will reconstitute skin but without appendage formation [21]. In 1993, Lichti developed a minimal component system consisting of newborn follicle epithelium and immortalized dermal papilla cell clones to test the hair-forming ability of selected cell populations. She found that hair follicle buds contribute to the formation of hairless skin when grafted alone or with Swiss 3T3 cells but produce densely haired skin when grafted with a fresh dermal cell preparation. Meanwhile, characterization of the phenotype of the dermal papilla cell lines, which differ in their ability to support hair growth when grafted with hair follicle buds, may provide insight into not only folliculoneogenesis and skin organ regeneration but also the effect of specific cellular and genetic manipulations on follicle growth [22]. Moreover, Iida's findings suggested that the hair-forming frequencies were affected by the hair cycle stages of both the follicle fragments and DPs. The follicle fragment containing the bulge (fragment III) at late anagen (LA) and the fragment between the bulge and hair bulb (fragment II) at catagen frequently generated hairs when associated with early anagen (EA)-DPs but infrequently with mid-anagen (MA)-DPs. Oppositely, anagen fragment II produced hairs at a high frequency with MA-DPs and at a low frequency with EA-DPs. Therefore, the hair inductivity of DPs can be altered between EA and MA, and follicular epithelial cells' proliferative activity would also change the stimuli-directed hair-forming ability of DP fragments [16]. In 2007, Osada et al. have succeeded in culturing dermal papilla (DP) cells long term and developed new techniques to enhance the hair follicle-inducing efficiency in a patch assay. More importantly, they aggregated dermal papilla cells to form spheres and then injected them with epidermal cells. The sphere formation was proven to be essential to model intact DP cells, resulting in not only hair follicle induction but also elevation of specific gene expression levels, even by later passaged cells [23]. In addition, the epithelial-mesenchymal signal

transmission in matured hair follicles exists not only in the dermal papilla but also in the surrounding connective tissue sheath (CTS), which also play a significant role in the formation and self-renewal of hair follicles [24].

20.4 Morphological Characteristics of Hair Follicle Cycling

20.4.1 Theories of Hair Follicle Cycling

In mammals, hairs do not persist throughout life but are periodically shed and regrown in a "hair cycle" consisting of three distinct phases: anagen, catagen, and telogen. In human scalp follicles, anagen, the growing phase, lasts several years; catagen, the involution phase, lasts approximately 1–3 weeks; and telogen, the resting phase, lasts for approximately 3 months. The cellular mechanisms involved in the maintenance of the phases and what triggers the transitions between them are poorly understood. In addition, human hair follicles grow in an asynchronous fashion. The majority (about 90%) of hair follicles are in the anagen phase, and less than 10% of follicles are in telogen, thus making it more difficult to study the structure and physiology of hair follicles. Recently, several pioneering studies have attempted to define the cellular dynamics of the hair follicle cycle that the DP cells express a cycle of released growth morphogens that orchestrate the cycle. Anagen switches on when the papilla morphogen concentration exceeds a critical threshold. However, the cell cycle-dependent fluctuation in papilla morphogens is inversely correlated with fluctuations in endogenous mitotic inhibitors. The follicle cycle is set up by the cell cycle of the DP cells that secrete morphogens only during the G0/G1 phase (papilla morphogen hypothesis) [25]. Moreover, Chase et al. held that an endogenous mitotic inhibitor accumulates during each anagen phase in the epithelial hair bulb [26]. When reaching for a certain threshold level, follicular cell growth would cease. However in telogen, the activity of endogenous mitotic inhibitors mentioned above would decrease to a level of disinhibition which contrarily triggers a hair to regrow, and the anagen phase starts again (inhibition-disinhibition hypothesis). Direct evidence for the theory is scant, although telogen epidermis has been reported to contain an inhibitor to hair growth induction, while anagen epidermis does not [27]. However, recent studies demonstrated that follicle formation is at least in part controlled by bone morphogenic protein-4/noggin complex—an inhibitor-release mechanism which encourages one to reassess Chase's inhibitor-release hypothesis for mature cycle initiation [28]. Furthermore, Cotsarelis in 1990 postulated that factors in the papilla instruct the stem cells of the bulge region to divide and orchestrate the hair cycling (bulge activation hypothesis) [29].

20.4.2 Bulge Activation Hypothesis of Hair Follicle Cycling

Coining Cotsarelis' hypothesis, Lavker and Sun et al. indicated that a signal from the DP stimulates bulge stem cells to divide and produce a new germinative hair

matrix which triggers the transition from a resting to a cycling follicle [30]. Actually, only the lower two-thirds portion of hair follicles undergoes a process of cycling [31]. The upper "permanent" portion of the hair follicle mainly consists of the sebaceous gland and arrector pili muscle. The hair follicle bulge which harbors epidermal multipotent stem cells is nestled below the sebaceous gland and marks the base of the "permanent" region of hair follicles. According to the bulge activation hypothesis, stem cells exit the bulge asymmetrically and then replenish the bulge stem cells and generate daughter TA stem cells upon induction of anagen in response to messages from the DP cells. Because of being less adhesive to the niche, the TA stem cells migrate down to regenerate the "cycling" portion of the hair follicle [32, 33]. Matrix cells proliferate and differentiate to generate the inner root sheath (cuticle and Huxley's and Henle's layers) and medulla and cortex cells, which together with the cuticle cells terminally differentiate into the mature hair fiber [19]. Histologically, anagen follicles are long and very straight and last approximately 2 weeks in mice and up to 4–5 years in humans. Then with the transition to catagen, the lower "cycling" portion of each hair follicle regresses entirely in a process that includes apoptosis of epithelial cells in the bulb and outer root sheath (ORS) and the outermost epithelial layer [34]. Meanwhile, the dermal papilla that is essential for follicle formation condenses and travels upward to the bulge where it remains during telogen. Interestingly, as the lower follicle recedes, a temporary structure forms—the epithelial strand—which is unique to catagen. This connects the DP to the upper part of the hair follicle, contains many apoptotic cells, and is completely eliminated by the time the DP reaches the cells that surround the bulge region of hair follicles [35]. Following catagen, follicles lie dormant in a resting phase (telogen). Subsequently, the transition from telogen to anagen occurs when the dermal papilla recruits stem cells of the bulge to commence a new process of proliferation and differentiation, thereby regenerating a new hair follicle [30]. Thus, the bulge activation hypothesis consists of the following basic components: (1) hair follicle stem cells are located in the bulge region of hair follicles; (2) the anagen stage of the hair cycle starts with the proliferation of bulge cells in response to a dermal papilla-derived inductive signal; (3) bulge cell proliferation is the cellular source of the entire hair follicle structure, including the hair matrix; (4) matrix cells are transit amplifying cells and have a limited proliferative potential that probably determines the length of anagen; and (5) the upward movement of the DP during catagen is crucial for re-establishment of DP-bulge-cell contact and consequent induction of a new hair cycle [36].

20.4.3 Role of Signal Factors in the Regulation of Hair Follicle Cycling

Although the actual signals that initiate and terminate the cycles of hair growth remain unclear, however, insights into the specific molecular mechanisms of hair follicle development and cycling have recently been made using animal models, which focus mainly on inductive pathways of anagen rather than on cellular

kinetics during follicle progression through anagen-catagen-telogen as the bulge activation hypothesis does. In 1998, Jindo analyzed the effect of cutaneous injections of recombinant human hepatocyte growth factor/scatter factor (HGF/SF) on hair follicle growth in different cycling stages and revealed that HGF/SF, a paracrine factor, can alter the cyclic hair growth of mice not only by delaying the transition from anagen to telogen of hair follicles but also by moderately inducing the initiation of anagen growth in hair follicles [37]. Subsequently, Lindner reconfirmed Jindo's result and demonstrated that endogenous HGF/SF and its receptor (Met) are involved in the regulation of hair follicle morphogenesis and cycling, which might be exploited for the therapeutic manipulation of human hair growth disorders [38]. In 1994, Paus et al. implied that IgE-independent mast cell (MC) secretagogues, compound 48/80 and ACTH, could induce anagen in mouse telogen follicles after intracutaneous administration, which was correlated with the occurrence of substantial MC degranulation by morphometry [39]. Thymosin β_4, an important mediator of cell migration and differentiation, recently was also reported to accelerate hair growth in normal rats and mice, due to its effect on critical events in the active phase of the hair follicle cycle, including promoting the migration of stem cells and their immediate progeny to the base of the follicle, differentiation, and extracellular matrix remodeling [40]. Moreover, neural cell adhesion molecule (NCAM), may play an important role in hair follicle topobiology during morphogenesis and cyclic remodeling [41]. NCAM was first detected on the developmental epithelial hair placodes and later on selected keratinocytes in the distal outer root sheath, whereas mesenchymal NCAM expression was noted on fibroblasts of the future dermal papilla (DP) and the perifollicular connective tissue sheath. Especially, fetal hair follicle elongation was coincided with strong, ubiquitous dermal NCAM expression, which remained strong until the follicles entered into their first neonatal catagen. However, as the hair follicle cycles consecutively, mesenchymal NCAM expression was seen exclusively on DP and perifollicular connective tissue sheath fibroblasts and on the trailing cells of regressing catagen hair follicles [41]. Therefore, the first hair cycle is distinct from all the subsequent hair cycles in their cellular origin and morphological sequence, which should be regarded as a neogenic event [42]. Another recently reported cell adhesion factor-related molecule involved in cutaneous biology is intercellular adhesion molecule-1 (ICAM-1), which is recognized for its pivotal role in mammalian inflammation and immune responses [43]. In the late stage of follicle morphogenesis, ICAM-1 expression in murine skin is widespread localizing in selected epidermal and follicular keratinocyte subpopulations, as well as interfollicular fibroblasts. Thereafter, morphologically identical follicles markedly differ in their ICAM-1 expression patterns, which become strikingly hair cycle-dependent in both intra- and extra-follicular skin compartments.

Furthermore, many of the signals involved in hair follicle formation in embryonic skin are also active during the telogen-to-anagen transition, when bulge cells are induced to produce a new hair follicle. For example, Sonic hedgehog (Shh) pathway, which is analyzed in exemplary organs including the feather, hair, tooth,

tongue papilla, lung, and foregut, is necessary for the regeneration and development of hair follicles. Transient, enhanced expression of the Sonic hedgehog (Shh) gene in postnatal skin would accelerate initiation of anagen in the hair follicle cycle and, at the same time, stimulate the dynamic transition from telogen to anagen, with consequent hair growth [44]. Meanwhile, the transcriptional control region of several hair-specific keratin genes each possesses a sequence motif that binds the Lef/Tcf family of HMG-box DNA-binding proteins [45]. Originally identified as a transcriptional regulator of T-lymphocyte differentiation, Lef1 (lymphoid enhancer factor 1) is also found in both the nuclei and cytoplasm of hair follicle matrix cells [46]. Elimination of Lef1 by gene targeting results in sparse hair, with a complete loss of whisker follicles and secondary follicles that account for the bulk of the hair coat. Conversely, transgenic misexpression of Lef1 in the interfollicular epithelium leads to occasional ectopic hair follicles [45]. Lef1 and its relatives are not ordinary transcription factors but, rather, DNA-binding proteins whose function is regulated by β-catenin, the downstream effector of canonical Wnt signaling.

In addition, several growth factor families, such as fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF)-I, and transforming growth factor-β (TGF-β) families, also appear to play pivotal roles in hair follicle growth [47–50]. The growth factors or cytokines mentioned above could mediate the activation of signal transducer and activator of transcription 3 (Stat3), which ultimately contribute to the initiation of a new anagen [51]. However, there are at least two distinct signaling pathways involved in wound healing and anagen progression of the hair cycling: Stat3-dependent pathway for spontaneous hair cycling and Stat3-independent pathway for exogenously induced hair cycling, whereas both pathways require phosphoinositide 3-kinase (PI3K) activation [52]. The molecular inductors for hair follicle growth and cycling have been summarized in Table 20.1.

20.5 Epidermal Stem Cell Compartments: Location and Capability for Multiple-Lineage Differentiation

In order to maintain homeostasis in the adult skin, epidermal stem cells are thought to divide infrequently, giving rise to short-lived (transit amplifying, TA) cells that undergo a limited number of cell divisions and ultimately terminal differentiation. This model for the epidermal stem cell niche has increased in complexity by the multiple populations of stem cells that have recently been identified to reside in different locations within the tissue, including the bulge [29, 53], interfollicular epidermis [54–57], sebaceous gland [58], and upper isthmus (UI) region of the HF [59], with each contributing to the generation of multiple lineages of skin cells. Recently, the defining characteristics of epidermal stem cell compartments in vitro have been studied extensively. There is evidence that under normal homeostatic conditions, the stem cells in different locations maintain the differentiated lineages that are appropriate for those locations [60, 61]. However, in response to injury or genetic

Table 20.1 Molecular inductors of hair follicle growth and cycling

Transcription factors	Location in follicle	Functions	Reference
Hepatocyte growth factor (HGF) HGF receptor, Met	Papilla Follicular bulb epithelium	Mediates E-M interactions, stimulates follicle growth in vitro Upregulation shows accelerated hair follicle morphogenesis by inducing anagen growth and retarded entry to catagen	[37] [38]
Sonic hedgehog (SHH)	Anagen bulb, IRS	Initiates anagen and stimulates the transition from telogen to anagen	[44]
Neural cell adhesion molecule (NCAM)	Developmental placode, ORS, DP, and connective tissue sheath	Thought to play regulatory role on the elongation of first hair cycling	[41, 42]
Intercellular adhesion molecule-1 (ICAM-1)	Epidermal and follicular keratinocyte subpopulations, interfollicular fibroblasts	Strikingly hair cycle-dependent patterning characteristics in both intra- and extra-follicular compartments	[43]
Lef-1	Hair follicle matrix cells, epithelium, and DP	Hair follicular morphogenesis	[45, 46]
Fibroblast growth factor (FGF)2 FGF receptor, FGFR1 FGF receptor, FGFR2	Follicular epithelial cell Papilla Matrix	Blocks follicle morphogenesis	[47]
Epidermal growth factor (EGF)		Follicle morphogenesis stimulates cell growth in the ORS but inhibits it in the matrix	[48]
Insulin-like growth factor (IGF)			
Insulin-like growth factor (IGF-1)	Bulb, ORS	Essential for follicle growth in vitro	[49]
Transforming growth factor-β (TGF-β)	Expressed in developing and matured follicles, IRS, ORS, and CTS	Plays a role in catagen development	[50]
Signal transducer and activator of transcription 3 (Stat3)		Stat3-dependent pathway for spontaneous hair cycling and Stat3-independent pathway for exogenously induced hair cycling	[51]

manipulation, different stem cell populations are functionally interconvertible. For example, the IFE is a stratified epithelium in which $K14^+$ proliferating cells are anchored to the basement membrane closest to the dermis. Experimental evidence showed that stem cells within the interfollicular epidermis can be reprogrammed to become hair follicle stem cells on sustained activation of WNT signaling [62, 63]. Although the ability of bulge stem cells to generate the seven different HF lineages underscores their multilineage potency [64], Oshima et al. provided that the bulge

region of adult mouse vibrissal follicles contained multipotent stem cells that respond to morphogenetic signals to differentiate into interfollicular epidermis, sebaceous gland, and HF lineages [33]. The bulge region of the HF has long been recognized as a heterogeneous population comprising a quiescent stem cell pool and stem cells that are rapidly activated for HF regeneration and wound response [29, 65, 66]. These cells have been purified based on the expression of the following marker molecules: CD34/α_6-integrin [67, 68], $a_6^{bri}CD71^{dim}$ [69], Lgr5 [70], keratin 15 (K15) [71], SRY box 9 (Sox9) [72], and label retention representing a slow-cycling keratinocyte population [29, 53]. Moreover, other genes that regulate ESCs and their fate in the HF bulge include GATA binding protein 3 (GATA3) [73], bone morphogenetic protein receptor1a (BMPR1a) [60], and the inhibitors of DNA-binding protein 2 and 4 (ID2, ID4) [74], Wnt and β-catenin [60, 73, 74]. Recent lineage-tracing experiments established that during normal skin homeostasis, bulge stem cells primarily contribute to HF compartments and the sebaceous gland, but not to the interfollicular epidermis [60, 61, 71]. In conditions such as wounding however, bulge stem cells rapidly migrate toward the interfollicular epidermis to help with the rapid regeneration of wounded skin [61, 71, 75, 76]. Using live cell imaging together with lineage-tracing experiments, Petersson et al. further showed that progeny of slow-cycling bulge cells (expressing CD34 and K15) migrate above the HF and follow a progression into the $Lrg6^+$, $Lrig1^+$ upper bulge region to the $Blimp1^+$ progenitor population, and the sebaceous gland to repopulate the mature sebocytes [77].

Additionally, stem and progenitor cell populations residing above the HF bulge have been identified. One stem cell population located in the UI of the HF is found to express the marker protein MTS24, also named as placenta-expressed transcript 1(Plet-1) [78]. However, this cell population does not express the HF bulge markers K15 and CD34 and is infrequently BrdU label retaining but is clonogenic in vitro [78]. Another stem cell population of the UI region expresses low levels of α_6-integrin and lacks CD34 and Sca-1 expression [59]. These stem cells are multipotent and possess a unique transcript profile compared with bulge stem cells. In a recent paper published in Science, Snippert et al. found that Lgr6, a close relative of the Lgr5 stem cell gene, marked a group of stem cells directly above the follicle bulge that generated cells of both the HF and the sebaceous gland during embryonic development [79]. Some cells of the interfollicular epidermis were also derived from Lgr6-expressing cells [79]. Like isthmus stem cells [59], Lgr6-expressing cells when transplanted into immunodeficient mice gave rise to all epidermal cell lineages. Moreover, like bulge stem cells, Lgr6-expressing stem cells were activated by wounding and migrated toward the epidermis to aid wound repair [79]. Taken together, these results lead the authors to conclude that Lgr6 marked the "mother" of all stem cells in the skin. However, it is not known whether or not the Lgr6 stem cell pool overlaps with the compartment lacking CD34 and Sca-1 expression. Moreover, a population positive for the transmembrane protein Lrig1, a marker for stem cells of the human interfollicular epidermis (IFE), constituted a multipotent stem cell compartment located in the junctional

zone between UI and IFE adjacent to the sebaceous glands [80]. These Lrig1-expressing cells can give rise to all of the adult epidermal lineages in skin reconstitution assays. However, during homeostasis and on retinoic acid stimulation, they are bipotent, contributing to the sebaceous gland and IFE, but not the HF lineages [80]. A further stem cell population that is present in the bulge and the isthmus is defined by the expression of GLI1, which is a transcription factor that is activated by Sonic hedgehog (SHH) from sensory neurons [81]. These GLI1-expressing cells were multipotent in their native environment and able to repeatedly regenerate the anagen follicle. They also migrated to the IFE on wounding, where they changed their lineage into epidermal stem cells and made a long-term contribution to the reepithelialized epidermis [69, 81]. The potential epidermal stem cell compartments in the skin are shown in Table 20.2.

Table 20.2 Epidermal stem cell compartments in the skin

Stem cells	Location	Characteristics	Multipotent capacity	Reference
Interfollicular stem cells	Basal layer of interfollicular epidermis	β1-integrin, K15, WNT, label retention	Contribute to interfollicular epidermal lineages in normal condition and presumably contribute to de novo hair follicle formation in the center of large excisional wounds	[53–55, 57, 62, 63]
Bulge stem cells	Bulge region of HF	CD34/α6-integrin, $a_6^{bri}CD71^{dim}$, Lgr5, K15, Sox9, GATA3, label retention, BMPR1a, ID2, ID4, Wnt, and β-catenin	Contribute to the HF compartments and sebaceous gland and respond rapidly to epidermal wounding	[29, 33, 60, 61, 65–77]
Isthmus stem cells	The upper part of HF, which interfaces with the interfollicular epidermis	One population expressing MTS24/Plet-1 The other expressing low levels of α_6-integrin but negative for CD34 and Sca-1	Clonogenic in vitro Give rise to epidermal, follicular, and sebaceous lineages and can self-renew in vivo	[78] [59]
Lgr6-expressing stem cells	Directly above the follicle bulge	Lgr6	Give rise to all epidermal cell lineages and can be activated to aid wound repair	[79]

Table 20.2 (continued)

Stem cells	Location	Characteristics	Multipotent capacity	Reference
Junctional zone stem cells	Junctional zone adjacent to the sebaceous glands and infundibulum	Lrig1	Generate all of the adult epidermal lineages in skin reconstitution assays. However, during homeostasis and on retinoic acid stimulation, mainly contribute to the sebaceous gland and IFE, but not the HF lineages	[80]
Gli1-expressing follicle stem cells	The bulge region and the isthmus	GLI1	Function as multipotent stem cells in their native environment and repeatedly regenerate the anagen follicle. Also migrate to the IFE on wounding to differentiate into epidermal stem cells	[81]

20.6 Progenitors Involved in Sweat Gland Regeneration

As an important appendage of the skin, the eccrine sweat gland was regarded for many decades as only having a key role in thermoregulation. However, that has changed. New and exciting avenues of research are elucidating novel information on sweat gland structure and functions. This has advanced our knowledge of human disorders associated with altered sweat gland activity, especially the utilization of sweat gland stem cells in improving wound healing (perhaps a key factor for diabetes) and being able to overcome temperature regulation issues in deep-burn survivors through the regrowing glands in the damaged skin.

Two distinct types of sweat gland exist in the human body: the eccrine or atrichial type (not connected to a hair follicle) and the apocrine or epitrichial type (linked to a hair follicle). The eccrine gland primarily consists of a coiled simple tubular structure that resides at the lower edge of the dermis and which connects to the skin surface via a straight intradermal portion and an intraepidermal segment. In human embryos, sweat gland buds begin to emerge on the palms and soles at 12–13 weeks and on the rest of the body at 20 weeks. At 22 gestational weeks of human, myoepithelial cells and luminal cells in the secretory portion can be detected. In the process of development, sweat gland germs grow down progressively into the dermis to form a duct, ending in a secretary coil, and play their function basing on the body itself and the changes in the external temperature. The mature sweat glands contain the portion of duct and secretary coil. Knowledge

of the eccrine sweat gland is relatively unified. In recent years, increasing evidence have shown that some stem cells are present in the sweat gland and play a role in the development and repair of sweat gland. Lu et al. and Van Keymeulen et al. recently pointed out the existence of two populations of stem cells in the sweat ducts of postnatal mice and four populations in the paw skin of adult mice [82]. Sweat glands begin to develop as a multipotent sweat bud progenitor ($K14^{+}$) during fetal life. After stratification, K18 expression is increased and K14 expression reduced, which generates a transient but proliferative suprabasal layer of progenitors ($K14^{low}/K18^{+}$). As development proceeds, these basal and suprabasal ductal progenitors continue to differentiate and migrate outward to form myoepithelial and luminal progenitor cells finally [83]. In adult paw skin, both luminal and myoepithelial progenitors in the secretory coil of sweat glands are capable of the maintenance of homeostatic turnover, and each followed distinct basal to suprabasal differentiation programs. Specifically, keratin expression profile analysis indicated that cells in the sweat glands express a number of characterized markers: luminal cells of a mature sweat gland express K8 and K18 [84]. NKAα, ATP1a1, and K19 are also expressed in the gland portion of a mature sweat gland [85, 86]. In contrast, K14 and K5 are found in the myoepithelial cells [87]. K10 is expressed positively in the duct portion of sweat gland cells [87]. Moreover, nestin, CD9, CD29, CD44, and CD81 were used to validate the stemness in the sweat glands [88].

20.7 Progenitor/Stem Cells and Sebaceous Gland Maintenance

The sebaceous gland plays a very prominent role in lubricating and waterproofing the epidermis, which requires constant replenishment to remain functional throughout life. However, the identification of sebaceous gland stem cells remains enigmatic. Early data reported that Blimp1 defines a population of sebaceous gland (SG) progenitors in mice [58]. However, it was subsequently rejected as a specific marker of sebaceous gland stem cells, due to its wide expression profile in the epidermis [89]. In addition, there is functional evidence to show that HF bulge stem cells can also contribute to SG maintenance in vivo. Fate mapping from a minimal K15 promoter and from the Lgr6 promoter driving Cre expression has demonstrated that stem cells residing within the bulge and the lower isthmus contribute to SG renewal [77, 79]. The cross-regulation between the bulge and sebaceous gland has also documented that Sox9-positive cells repopulating the future HF bulge region are involved in SG maintenance [76]. However, recent lineage-tracing data using Lrig1, which marks basal cells in both the junctional zone and the sebaceous gland, strongly support the contention that basal cells within the sebaceous gland form an autonomous source for cellular replenishment and that the sebaceous gland is maintained independently of all other compartments [90].

20.8 Other Stem Cell Lineages in the Skin

20.8.1 Mesenchymal Stem Cells

In the last two decades, skin stem cell biology has been a rapidly advancing field in the life sciences. The understanding that the skin as a whole represents a larger reservoir of adult stem cells (including mesenchymal, hematopoietic, and neural stem cells) than the epidermis alone has increased tremendously [91]. These pluripotent stem cells have evoked significant excitement because of their potential for therapeutic applications, especially in skin injury from burns or exposure to hazardous chemicals, and major healthcare problems because of an increasing number of industry-related accidents worldwide. To utilize stem cells for grafting and skin tissue regeneration, we must develop well-defined and efficient resources of embryonic stem cells derived from the inner cell mass of embryos at the blastocyst stage, putative epidermal stem cells located at the bulge of hair follicles, or even adult stem cells of non-epidermal origin, such as that derived from the bone marrow and peripheral blood circulation, which are capable of transdifferentiation into a keratinocyte lineage.

A number of factors would favor the use of embryonic stem cells over adult stem cells for the repair and regeneration of skin tissues. The most obvious is their capacity for immortality and self-renewal, which enables them to provide an unlimited supply of differentiated keratinocytes or keratinocyte progenitors for treating skin injury. However, since the 1970s, embryonic stem-cell-related research has raised a host of difficult ethical issues and has sparked great public interest and controversy. By contrast, adult stem cells derived from the skin, bone marrow, or peripheral blood, with their limited capacity for self-renewal and proliferation, which may decrease with age, would be more acceptable for therapeutic application in human skin tissues. For example, the potential role of mesenchymal stem cells (MSCs) in cutaneous wound healing has also been gradually excavated not only because of their remarkable plasticity but also due to their ability to home and engraft into damaged tissues [92] and low immunogenicity [93]. MSCs can be identified by the expression of many molecules including CD73, CD105, and CD90 and no expression of CD45, CD34, CD14 or CD11b, and CD79ūFC; or CD19. Several preclinical and clinical studies have shown that autologous or allogeneic MSCs are safe and therapeutic in the treatment of chronic wounds [94–96], burn injuries [97, 98], surgical wounds [99], and limb ischemia [100]. These cells can regulate the function of inflammatory cells, such as macrophages, neutrophils, and T cells, so as to hasten the healing of wounds by triggering an anti-inflammatory response [101]. Subsequently, MSCs can be directed to differentiate into multiple skin cell lineages, including keratinocytes [102], adipocytes [103], and endothelial cells (ECs) [104], and secrete a variety of cytokines to promote wound reepithelialization and limit excessive scarring [101, 105, 106]. Furthermore, MSCs, in response to the host environment, can be recruited to the site of injury to induce neovascularization [107], increase cell migration and proliferation [108],

and affect the metabolic activity of host cells and tissues [109]. Particularly, many of the effects of MSC treatment are thought to be due to the release of soluble factors that regulate the local cellular response to injury [110, 111], affecting multiple signaling pathways.

20.8.2 Hematopoietic Stem Cells

Meanwhile, hematopoietic stem cells (HSCs) are a blood cell progenitor cell that gives rise to all the blood cells through hematopoiesis. There is little literature discussing the use of purified HSCs as a wound repair strategy, but therapies that utilize bone marrow aspirates no doubt contain a mixture of MSCs, HSCs, and a range of other multipotent progenitor cells. It is understood that hematopoietic and mesenchymal progenitor cells mobilize from the bone marrow into the pool of circulating cells after tissue injury. Among these cells, HSCs travel to the skin and aid in the regeneration of damaged epithelium. A clinical trial conducted by Wettstein et al. investigated the effect of using autologous HSCs in chronic wounds in a pressure sore model [112]. Three patients underwent cell harvest from the iliac crest at the time of the initial debridement, and the $CD34^+$ cells were selected and injected as a cell suspension into the wound bed [112]. The results indicated that there was a decrease in wound size on the cell-treated side. Although this result was not statistically significant, a 2-year follow-up examination revealed no signs of malignancy in the area of treatment [112].

In our laboratory, we observed that CD34, a specific hematopoietic stem cell (HSC) marker, was expressed in the cytoplasm and membrane of a small fraction of human epidermal stem cells [113]. These cells were round shaped with kidney-like nuclei and exhibited a high nucleus-to-cytoplasm ratio, which were similar to the morphological features of blood mononuclear cells. To further investigate the cell origins during skin development, we detected the expression of CD34 and another hematopoietic stem cell surface marker CD133 in skin tissues by immunohistochemical staining during various stages of human embryogenesis. Although CD34 staining was negative in the epidermis, it was detectable with low-level expression in the dermal fibroblasts and high-level expression in the follicle matrix cells [113]. The expression of CD133 was significantly correlated with the expression of CD34 [113]. The matrix contains actively dividing, relatively undifferentiated cells and surrounds the dermal papilla, a pocket of mesenchymal cells essential for follicle formation. The cell-matrix interaction allows dermal papilla cells to contact epithelial hair matrix cells, an important relationship that appears to play a role in reciprocal epithelial-mesenchymal cross talk and is crucial for normal development of hair follicles, as well as for hair cycling. Our observation suggested a key role of HSCs in the molecular control of epithelial-mesenchymal cell interactions. The mutual cross talk between epithelial and mesenchymal cells is crucially involved in the tight control of keratinocyte proliferation and differentiation and of vital importance during cutaneous wound healing and reepithelialization.

20.8.3 Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) can be isolated from peripheral blood or bone marrow. There are a wide range of surface markers that can be used to characterize EPCs, whereas they are generally agreed to be $CD34^+$, $CD133^+$, and VEGFR-2^+ [114]. Given that EPCs act as endothelial precursors that promote new blood vessel formation and increase angiogenesis by secreting growth factors and cytokines in ischemic tissue, it then makes sense that EPC should accelerate the wound repair process by facilitating neovascularization and the production of various molecules related to wound healing. Chronic wounds, e.g., leg and foot ulcers, are often accompanied by a compromised vasculature. Therefore, proangiogenic EPCs are a logical therapeutic target. Several publications including preclinical and clinical studies have reported that EPC transplantation accelerated wound healing by enhancing neovascularization in granulation tissue [115]. In a murine dermal excisional wound model, the author showed that the intradermally injected EPCs secreted a variety of wound healing-related chemoattractants, thereby promoting the recruitment of monocyte/macrophage and stimulating endogenous angiogenesis during the wound healing process [116]. Another study also described the use of a biodegradable RGD-g-PLLA scaffold to deliver EPCs to a murine wound model. This study indicated that target delivery of transplanted EPCs resulted in improved cell survival over their conventional local injection method [117]. Di Santo et al. further showed that the intramuscular injection of EPC-derived conditioned medium (EPC-CM) was equivalent to cell transplantation for promoting tissue revascularization and functional recovery, suggesting that EPC-CM might serve as another therapeutic option that is free from allograft-associated immune rejection concern [118]. These results suggested that EPC transplantation could be beneficial for the treatment of cutaneous wounds, especially chronic wounds that are often associated with decreased peripheral blood flow and remain difficult to heal using current therapeutic approaches.

20.8.4 Skin-Derived Neural Stem Cells

Skin-derived neural precursor cells (skin-NPCs) were isolated and characterized as skin-derived precursors (SKPs) in 2001. SKPs are a distinct population of skin stem cells which exhibit properties of neural crest (NC) precursors. These precursors migrated into the skin during embryogenesis and maintained their multipotency until adulthood like their NC ancestors [119]. Similar to their potential developmental origin, the SKPs can generate both neural and mesodermal progeny and differentiate into the separate subpopulation of cells expressing neuronal, glial, smooth muscle, adipocyte, and osteoblast markers [120]. For instance, adult neural stem cells, obtained from the skin and in the presence of transforming growth factor-β during colony formation, might be an accessible source of autologous cell replacement therapy. Dermis-derived progenitor cells transplanted into the spinal cord after traumatic injury migrated to the lesion site and differentiated into cells expressing

glial and neuronal markers. Therefore, neural stem cells from the skin could be the basis for a novel and potential therapeutic strategy for treating disease and trauma of the nervous system.

20.9 Plasticity of Skin Stem Cells in Cutaneous Repair and Regeneration

20.9.1 Transdifferentiation Capacity of Epidermal Stem Cells

As mentioned above, epidermal stem cells play a key role in maintaining the physiological function and homeostasis of skin tissues, and they are also a useful model for regenerative medicine. Recent studies have shown that somatic epidermal stem cells have a wide developmental capacity and the ability to produce cells from a diverse number of tissues in both mouse and chick. For example, Yu et al. reported that a population of cells in the HFs that proliferated as spherical aggregates expressed nestin and could differentiate into multiple lineages [121]. Uchugonova et al. further showed that the bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle [122]. The nestin-expressing stem cells migrate from the bulge area to the dermal papilla (DP) as well as into the surrounding skin tissues including the epidermis and during wound healing [122]. Nestin is a cytoskeleton-associated class VI intermediate filament protein (N-1), the expression of which was first identified in nervous system progenitor cells. Nestin expression has also been reported in non-neuronal tissues, including embryonic and adult tissues, in vivo and in vitro. Nestin-expressing cells show characteristic features of proliferation, migration, and a broad differentiation potential into mesenchymal/mesodermal, neural, pancreatic endocrine, as well as hepatic cell lineages [123, 124]. By using green fluorescent protein (GFP) tracing techniques in mice, Amoh et al. observed that the pluripotent nestin-driven GFP-tagged stem cells were positive for the stem cell marker CD34 and could differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro [125]. When the GFP-tagged HF stem cells were transplanted into the gap region of a severed sciatic nerve, they promoted nerve regeneration, leading to the restoration of nerve function [125]. After transplantation to severed nerves, however, the HF stem cells differentiated largely into Schwann cells [126], which are known to support neuron regeneration.

Moreover, keratinocytes together with fibroblasts arrayed on a three-dimensional matrix are capable of supporting the development of functional human T cells from hematopoietic precursors in the absence of the thymic tissue [127]. In a subsequent study, Nijhof et al. demonstrated that MTS24/Plet1, a marker of thymic epithelial progenitor cells, also identified a novel population of follicular stem cells located between the bulge and the sebaceous glands [78]. These data suggest that potentially, functional and phenotypic links may exist between progenitor cells in epidermal and thymic epithelia.

20.9.2 Dedifferentiation Capacity of Epidermal Stem Cells

The cellular plasticity and reversibility observed in adult epithelial tissues have not been associated with "transdifferentiation" into completely unrelated fates but rather with contribution to the repair of the tissue from which the cells originated. In this regard, the plasticity seems to arise through a process of dedifferentiation and/or redifferentiation. Accumulating evidence now shows that adult epidermal keratinocyte stem cells can be reprogrammed to become similar to embryonic stem cells. Liang et al. demonstrated that mouse epidermal stem cells, when injected into a blastocyst, produced different tissues with origins in all three of the germ cell layers [128]. Li et al. reported the use of nuclei from bugle stem cells as nuclear transfer donors and cloned healthy and viable mice that survived until adulthood [129]. Strikingly, a paper published in *Nature* showed that keratinocytes from the outer layers of the skin could reprogram much more efficiently than fibroblasts [130]. In parallel experiments using the same batch of retroviral supernatants (Oct3/4, Sox2, Klf4, and c-Myc), the infection of keratinocytes from juvenile human foreskin yielded iPSCs at an efficiency of ~1%. By contrast, infection of fibroblasts obtained from the same skin sample yielded an overall reprogramming efficiency of <0.01%, in agreement with previous reports [130]. Moreover, the keratinocyte-derived iPSCs emerged just 10 days after infection [130], as compared to 21–25 days after transduction in the case of the fibroblasts. It therefore appears that skin keratinocytes are more readily reprogrammable than fibroblasts. The mechanisms underlying why epidermal keratinocyte stem cells were relatively easy to reprogram is unknown. However, Aasen et al. hypothesized that epidermal keratinocytes might have a transcriptional and epigenetic state that is more favorable to reprogramming [130]. For instance, Myc, which is detected in the basal layers of the epidermis [131], the proliferative zone at the base of the follicle (bulb), the quiescent zone of stem cells in the bulge and in the terminally differentiating matrix cells that lie above the bulb and gave rise to the hair fiber, plays a key role in homeostasis of the skin [132]. Gandarillas et al. showed that Myc was necessary to drive epidermal stem cells to a transit amplifying state and to undergo terminal differentiation [133]. Gebhardt et al. further pointed out that Myc regulated keratinocyte adhesion and differentiation by binding to Miz1, a zinc-finger protein mediating Myc repression of gene expression [134]. In analogy to Myc protein, Klf4 is another reprogramming-related transcription factor required for epidermal stem cell differentiation [135]. Therefore, epidermal keratinocytes contain higher endogenous levels of Klf4 and c-Myc transcripts compared to fibroblasts, which may explain in part why they are more amenable to reprogramming. It is also conceivable that epithelial cells per se are more amenable to reprogramming because unlike fibroblasts, they are not required to undergo a mesenchymal-to-epithelial transition to give rise to embryonic stem cells [130, 136]. Moreover, a recent study demonstrated that hair follicle DP cells that endogenously express high levels of Sox2 and c-myc could be reprogrammed into iPSC state with only Oct4 and Klf4, suggesting a convincing experimental

evidence of the molecular basis for the broader ability of epidermal stem cells [137]. The differentiation and transdifferentiation ability of epidermal keratinocyte stem cells are summarized in Table 20.1.

20.9.3 Regulation of Cellular Plasticity in Epidermal Stem Cells

The skin epidermis and its appendages (e.g., hair follicles, sebaceous glands, and sweat glands) harbor spatially distinct stem cell pools. Given that the skin serves as a protective barrier against the outside world, the skin stem cells play a crucial role in maintaining tissue homeostasis by providing new cells to replace those that are constantly lost during tissue turnover or following injury. Recently, the defining characteristics of epidermal stem cell compartments in vitro have been studied extensively. These studies showed that the fate and multilineage potential of epithelial stem cells can broaden and be modulated depending on whether a stem cell exists within its resident niche and responds to normal tissue homeostasis, whether it is mobilized to repair a wound, or whether it is taken from its niche and challenged to de novo tissue morphogenesis after transplantation [64]. For example, it has been argued that bulge stem cells were multipotent based on their ability to be mobilized and differentiate to the IFE upon injury or induction of inflammation [32, 53, 138], whereas replacement of damaged hair follicle compartments with IFE-derived cells has been difficult to perform due to the inability to efficiently eliminate parts of the PSU [61]. However, lineage-tracing experiment using laser ablation of bulge stem cells has recently showed that cells from the upper PSU or the IFE can migrate down to the lower PSU and replace bulge stem cells [139, 140], whereas the ablated bulge cells can be also replenished by the cells from underlying hair germ [141]. Then, we argued what causes the lineage infidelity and promotes the breakdown of plasticity barrier. The concept of niche vacancy gave an explanation that surrounding stem cell progenies or stem cells from a more distant niche can home in to fill the vacant niche upon local disturbance and loss of stem cells [141]. Meanwhile, ablation of a myoepithelial cell of the sweat gland prompted rescue by a dividing neighboring myoepithelial cell but not a luminal or ductal cell [142]. In consistence with this observation, ablation of a luminal cell was rescued only by its own lineage [142]. Intriguingly, such boundaries can break down, as shown in transplantation experiments in which a purified myoepithelial cell upon engraftment into a cleared mammary fat pad could regenerate an entire sweat gland [142] providing a corroborative evidence for the impact of the microenvironment on stem cell fate change and destination. Further, it was recently demonstrated that heightened stress, such as wounding and transplantation, induced the activation of a panel of transcription factors and drove the onset of lineage infidelity by remodeling stress-specific regulatory elements, leading to suppression of certain stem cell identity genes and to activation of other genes [141]. In this work, the author pointed out that lineage infidelity occurs in wounding when stress-responsive enhancers become activated and override homeostatic enhancers that govern lineage specificity [141]. Specifically, the collaboration between lineage and stress factors activated oncogenic enhancers that distinguish cancers from wounds [141].

20.10 Contributions of Epidermal Stem Cells to Cancer

Given that stem cells have the highest probability of acquiring the multiple genetic alterations required for tumorigenesis, a great deal of evidence in the mouse system points to stem cell populations in the different PSU compartments as targets of carcinogenesis. For example, aberrant accumulation of altered stem cells in hair follicles and their subsequent migration to the epidermis contribute to human papillomavirus (HPV)-induced tumor development [70]. Basal cell carcinomas (BCCs) are thought to arise from stem cells that normally reside in the bulge of the hair follicle, and that wound microenvironment stimulates stem cell migration and tumor promotion [143–145]. Meanwhile, expression of the HMG-box-containing transcription factor Sox9 has been shown to be essential for the development of the hair follicle bulge stem cell compartment [146], and activated Sox9 is constitutively maintained in the epithelial compartment of cutaneous neoplasms including BCC, trichoepitheliomas, and trichilemmomas [147]. A paper published in *Cell* also identified a molecule called Yap1—the transcriptional effecter of the Hippo signaling pathway—as a critical modulator of epidermal stem cell proliferation and tissue expansion [148]. Using gain- and loss-of-function studies, the authors show that Yap1 mediates this effect through interaction with TEAD transcription factors. And, α-catenin is one of the upstream negative regulators of Yap1. Since α-catenin is a molecule previously implicated in tumor suppression and cell density sensing in the skin, these findings underscore the link between skin regeneration and cancer development [148].

Several crucial pathways, such as Hedgehog and WNT signaling that are important regulators of epidermal stem cell evolution and homeostasis, are also involved in the epithelial cancers. In humans, activating mutations in β-catenin have been found in pilomatricomas and trichofolliculomas, whereas mutations in the amino terminus of lymphoid enhancer-binding factor 1 (LEF1) that block β-catenin binding are found in human sebaceous gland tumors [149]. In mice, inducible activation of β-catenin under the control of the K14 promoter leads to the formation of lesions resembling pilomatricomas that regress when β-catenin is no longer activated [150]. Expression of dominant negative mutant transcription factor Lef1 (ΔNLEF1) via the K14 promoter results in the formation of sebaceous gland tumors [151]. Ablation of β-catenin expression directed by the K14 promoter results in the complete regression of chemically induced murine tumors [152]. Interestingly, different epidermal stem cell populations exhibit differing sensitivity to WNT-associated tumor formation [153]. Sustained activation of β-catenin, under the control of the K15 promoter, stimulated bulge expansion and caused the existing hair follicles to enter anagen, but it was not sufficient to induce pilomatricomas, even when combined with wounding [154]. However, prolonged activation of β-catenin under the control of a truncated K5 (ΔK5) promoter, which was expressed in cells at the base of sebaceous gland, leads to the conversion of the sebaceous gland into hair follicles, which subsequently overgrow and resemble benign tumors [154]. It is evident that members of the WNT signaling pathway play a significant role in maintaining the phenotype of epidermal stem cells and in the commitment of stem cells to differentiate along

the hair or interfollicular/sebocyte lineages. It appears that varying the strength, timing, and duration of an individual molecule within the signaling pathway exerts different effects on the epidermal stem cell compartment [62, 74].

Hedgehog is another key factor whose expression must be temporally regulated to ensure epidermal stem cell homeostasis. Mutations in the Shh signaling pathway are most notably associated with the development of certain skin cancers such as BCCs [155]. In mice, the Hedgehog pathway can be activated in several different ways, including deletion of Ptch1, overexpression of Gli1 or Gli2, or mutational activation of the signaling effector smoothened (Smo). Loss-of-function Ptch1 mutations or activating mutations in Smo have been detected in sporadic BCCs and in the nevoid BCC syndrome in humans [123]. Shh target genes Gli1 and Ptch1 are upregulated in nearly all BCCs examined [156], and the overexpression of Shh, Gli1, or Gli2 in mice also leads to BCC and other hair follicle-derived tumors [157, 158]. Just as in the case of WNT signaling, the originating cell can also influence the subtype of BCC that develops in response to different domains and levels of Hedgehog activation. By expressing a constitutively activated mutant of SMO to activate Hedgehog signaling in different subsets of cells of the skin epidermis, Youssef et al. found that BCCs arose preferentially from cells in the IFE and the infundibulum rather than from cells in the bulge [144]. In a different study, Wang et al. found that X-ray-induced BCC in Ptch1$^{(\pm)}$ mice originated from the keratin 15-expressing stem cells of the follicular bulge [159]. Conditional loss of p53 not only enhanced BCC carcinogenesis from the bulge but also produced BCCs from the interfollicular epidermis as a result of enhanced SMO expression [159]. In addition to the direct enhancement of tumor growth, the activation of Hedgehog signaling in combination with wounding can induce the movement of stem cell progeny to a new location to initiate tumorigenesis [143, 145]. Overall, these studies highlight the impact of targeting genes unique for stem cell behavior maintenance, particularly self-renewal, on tissue homeostasis and tumorigenesis and collectively indicate that genes unique to epidermal stem cells are potentially the most effective targets for cutaneous cancer treatment and prevention.

20.11 Clinical Applications of Epidermal Stem Cells

Major skin injuries, resulting from extensive burns, infection, or trauma, cannot repair alone and require medical intervention to heal properly. Autologous skin grafting, consisting of the removal of a piece of skin from unaffected tissue and its transplantation to the wounded area, is the most viable and aesthetically pleasing technique for the treatment of extensive skin injuries. Nevertheless, this approach has important limitations; for example, only a limited fraction of the skin can be repaired by this method, and it creates additional injuries at the donor sites. For these reasons, scientists search for alternative methods to treat severe skin injuries. Adult epidermal stem cells, in both the epidermis and the HF, can form colonies in vitro and have a high expansion capacity to reform a functional skin barrier that can be transplanted into patients suffering from severe and extensive burn injuries.

There is also growing evidence that the keratinocyte stem/progenitor cell has a unique immunological profile and displays immune privilege: the virtual absence of MHC class I expression [160] and low numbers of immune cells in the HF have been observed [161]. This immune privilege of epidermal stem/progenitor cells offers the potential for their use as universal donors in the cell-based treatment of full-thickness skin injury.

Epidermal stem cells also have the potential to treat many other afflictions, including inherited skin disease, cardiac disease, spinal cord and brain injury, degenerative diseases such as multiple sclerosis and Alzheimer's, diabetes, and immune disease [123, 162]. Importantly, in 2007, a major breakthrough came with the demonstration that exogenous addition of only four transcription factors (Oct4, Sox2, Myc, and Klf4) was able to reprogram mature differentiated cells, including fibroblasts [163], keratinocytes [130], lymphocytes [164], or liver cells [136], into pluripotent embryonic stem cells, with the ability to differentiate into multiple types of specialized cells of the body. More recently, iPSC-derived fibroblasts and keratinocytes from patients with recessive dystrophic epidermolysis bullosa were designed to generate 3D skin equivalents and rebuild human skin histological structure on the backs of mice [165]. These findings open new avenues for the generation of patient-specific pluripotent stem cells and redifferentiation of these cells into various defective or damaged cell types. However, the longtime goal in regenerative medicine is to produce specialized reparative cells directly from a pool of adult cells that are healthy, abundant, and easily obtained. Until now, reparative cells have been generated from embryonic stem cells and more recently from pluripotent stem cells created from fully reprogramming adult cells. As mentioned above, iPSCs represent the combined advantages of the pluripotency of ESCs and the availability of MSCs. However, there are still numerous issues, such as their risk for tumorigenicity in an undifferentiated state. It thus makes sense that populations exhibiting sufficient plasticity to switch to a desired lineage, but not a fully pluripotent lineage, may serve as a more practical and safe alternative to iPSCs for autologous cellular replacement therapies.

A technique described in *Nature* has shed some light on the possibilities for directly reprogramming somatic cells into other mature cell types [166]. By injecting a cocktail of three viruses carrying key regulatory genes in beta-cell development, Zhou et al. converted adult pancreatic exocrine cells into insulin-producing beta-like cells [166]. The reprogrammed cells were similar to beta cells in appearance, size, and shape; expressed genes characteristic of beta cells; and were able to partially restore blood sugar regulation in mice whose own beta cells had been chemically destroyed [59]. Zhou's experiment provided a template for direct lineage conversion between distantly related cell types. Based on this work, a slew of papers have confirmed and demonstrated that this kind of cell transformation can also be achieved in monocytes [167], cardiomyocytes [168, 169], and neurons [170, 171] by using a well-controlled process of genetic modification. The recent studies on lineage reprogramming have revealed several common themes, despite the differences in cell types involved. The most striking of these themes is that, in almost all cases, the reprogramming factors identified to date were transcription factors.

Although the modulation of signaling pathways would be an attractive alternative to transcription factors because lineage conversion could be achieved without genetic modification, it seems that modulation of signaling pathways is able to induce transdetermination but would be insufficient to induce the conversion between more distantly related lineages [172]. The predominance of transcription factors in reprogramming experiments is not surprising given that they are the primary effectors of lineage decisions during normal development. And, forced expression of lineage-specific transcription factors would be more likely to be sufficient to induce the necessary transcriptional and epigenetic changes for generating distinct cellular fates de novo [173]. With this idea, Mauda-Havakuk et al. reported that ectopic expression of transcription factors that control pancreas organogenesis such as PDX-1 could activate the pancreatic lineage and function in ectoderm-derived human keratinocytes [174].

Mammalian skin is a highly accessible tissue source for adult stem cells. The greater potential of epidermal stem cells to be reprogrammed to a wide spectrum of cell types, together with their enormous expansion potential in vitro, suggests that the epidermal stem cells obtained from a patient's own skin could be the ideal source of cells for direct cellular reprogramming into the desired committed lineages that are defective or missing in human disease, thereby avoiding issues of histocompatibility.

20.12 Conclusions and Perspective

Studies on the skin stem cell compartment have important implications in both basic research and regenerative medicine. Especially, lineage switching via direct conversion, without passing through a pluripotent state, offers exciting new experimental tools for developing patient-specific, regenerative medicine approaches. If this technique can be replicated in humans, it may be possible to convert a wide range of adult cells to other cell types using a small number of regulatory genes. Several important questions must be addressed: (1) How do epidermal stem cells evolve during development and how are they maintained in the adult? Before switching to other cell lineages, substantial research and development is required to address the molecular mechanisms that control epidermal stem cell behavior, proliferation, and differentiation. (2) If epidermal stem cells have the capacity to be reprogrammed, how flexible are the fates of these cells? Given the myriad of complex molecular communications that take place within the niche to coordinate stem cell activity during homeostasis and wound repair, new technological revolutions should be developed for our better understanding on the nature of distinct epidermal stem cell niches and to elucidate how spatial and temporal niche information impinge on the changes in behavior and plasticity when epidermal stem cells are confronted with a wounded state. And (3) more attention should be paid to the cell fate conversion process of epidermal stem cells to ensure safety and functionality in transplanted cell populations for biomedical applications. In particular, the roles of translational regulation, noncoding RNAs, and metabolism in contributing to these dynamics

should be further explored. The better we understand epidermal stem cell plasticity, the more we will be empowered with the full potential of epidermal stem cell for regenerative medicine. This knowledge should also enable us to develop an elaborate regulatory method to accelerate epidermal repair and generate patient-specific cell types of interest while minimizing cancer risk.

References

1. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT. Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen. 2009;17:763–71.
2. Gottrup F. A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am J Surg. 2004;187:38S–43S.
3. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet. 2005;366:1736–43.
4. Martin P. Wound healing--aiming for perfect skin regeneration. Science. 1997;276:75–81.
5. Stojadinovic O, Brem H, Vouthounis C, Lee B, Fallon J, Stallcup M, Merchant A, Galiano RD, Tomic-Canic M. Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing. Am J Pathol. 2005;167:59–69.
6. Stojadinovic O, Pastar I, Vukelic S, Mahoney MG, Brennan D, Krzyzanowska A, Golinko M, Brem H, Tomic-Canic M. Deregulation of keratinocyte differentiation and activation: a hallmark of venous ulcers. J Cell Mol Med. 2008;12:2675–90.
7. Nakamura M, Tokura Y. Epithelial-mesenchymal transition in the skin. J Dermatol Sci. 2011;61:7–13.
8. Hudson LG, Newkirk KM, Chandler HL, Choi C, Fossey SL, Parent AE, Kusewitt DF. Cutaneous wound reepithelialization is compromised in mice lacking functional Slug (Snai2). J Dermatol Sci. 2009;56:19–26.
9. Savagner P, Kusewitt DF, Carver EA, Magnino F, Choi C, Gridley T, Hudson LG. Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes. J Cell Physiol. 2005;202:858–66.
10. Dhouailly D, Olivera-Martinez I, Fliniaux I, Missier S, Viallet JP, Thelu J. Skin field formation: morphogenetic events. Int J Dev Biol. 2004;48:85–91.
11. Launay C, Fromentoux V, Shi DL, Boucaut JC. A truncated FGF receptor blocks neural induction by endogenous Xenopus inducers. Development. 1996;122:869–80.
12. Fuchs E. Scratching the surface of skin development. Nature. 2007;445:834–42.
13. Alonso L, Fuchs E. Stem cells in the skin: waste not, Wnt not. Genes Dev. 2003;17:1189–200.
14. Ellis T, Gambardella L, Horcher M, Tschanz S, Capol J, Bertram P, Jochum W, Barrandon Y, Busslinger M. The transcriptional repressor CDP (Cutl1) is essential for epithelial cell differentiation of the lung and the hair follicle. Genes Dev. 2001;15:2307–19.
15. Paus R, Muller-Rover S, Van Der Veen C, Maurer M, Eichmuller S, Ling G, Hofmann U, Foitzik K, Mecklenburg L, Handjiski B. A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. J Investig Dermatol. 1999;113:523–32.
16. Iida M, Ihara S, Matsuzaki T. Hair cycle-dependent changes of alkaline phosphatase activity in the mesenchyme and epithelium in mouse vibrissal follicles. Develop Growth Differ. 2007;49:185–95.
17. Kumar A, Velloso CP, Imokawa Y, Brockes JP. Plasticity of retrovirus-labelled myotubes in the newt limb regeneration blastema. Dev Biol. 2000;218:125–36.
18. Combates NJ, Chuong CM, Stenn KS, Prouty SM. Expression of two Ig family adhesion molecules in the murine hair cycle: DCC in the bulge epithelia and NCAM in the follicular papilla. J Investig Dermatol. 1997;109:672–8.

19. Hardy MH. The secret life of the hair follicle. Trends Genet. 1992;8:55–61.
20. Kobayashi K, Nishimura E. Ectopic growth of mouse whiskers from implanted lengths of plucked vibrissa follicles. J Investig Dermatol. 1989;92:278–82.
21. Weinberg WC, Goodman LV, George C, Morgan DL, Ledbetter S, Yuspa SH, Lichti U. Reconstitution of hair follicle development in vivo: determination of follicle formation, hair growth, and hair quality by dermal cells. J Investig Dermatol. 1993;100:229–36.
22. Lichti U, Weinberg WC, Goodman L, Ledbetter S, Dooley T, Morgan D, Yuspa SH. In vivo regulation of murine hair growth: insights from grafting defined cell populations onto nude mice. J Investig Dermatol. 1993;101:124S–9S.
23. Osada A, Iwabuchi T, Kishimoto J, Hamazaki TS, Okochi H. Long-term culture of mouse vibrissal dermal papilla cells and de novo hair follicle induction. Tissue Eng. 2007;13: 975–82.
24. Ito M, Sato Y. Dynamic ultrastructural changes of the connective tissue sheath of human hair follicles during hair cycle. Arch Dermatol Res. 1990;282:434–41.
25. Stenn KS, Paus R. What controls hair follicle cycling? Exp Dermatol. 1999;8:229–33. discussion 233-226.
26. Chase HB. Growth of the hair. Physiol Rev. 1954;34:113–26.
27. Paus R, Stenn KS, Link RE. Telogen skin contains an inhibitor of hair growth. Br J Dermatol. 1990;122:777–84.
28. Botchkarev VA, Botchkareva NV, Roth W, Nakamura M, Chen LH, Herzog W, Lindner G, McMahon JA, Peters C, Lauster R, McMahon AP, Paus R. Noggin is a mesenchymally derived stimulator of hair-follicle induction. Nat Cell Biol. 1999;1:158–64.
29. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 1990;61:1329–37.
30. Sun TT, Cotsarelis G, Lavker RM. Hair follicular stem cells: the bulge-activation hypothesis. J Investig Dermatol. 1991;96:77S–8S.
31. Fuchs E, Merrill BJ, Jamora C, DasGupta R. At the roots of a never-ending cycle. Dev Cell. 2001;1:13–25.
32. Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell. 2000;102:451–61.
33. Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell. 2001;104:233–45.
34. Lindner G, Botchkarev VA, Botchkareva NV, Ling G, van der Veen C, Paus R. Analysis of apoptosis during hair follicle regression (catagen). Am J Pathol. 1997;151:1601–17.
35. Alonso L, Fuchs E. The hair cycle. J Cell Sci. 2006;119:391–3.
36. Panteleyev AA, Jahoda CA, Christiano AM. Hair follicle predetermination. J Cell Sci. 2001;114:3419–31.
37. Jindo T, Tsuboi R, Takamori K, Ogawa H. Local injection of hepatocyte growth factor/scatter factor (HGF/SF) alters cyclic growth of murine hair follicles. J Investig Dermatol. 1998;110:338–42.
38. Lindner G, Menrad A, Gherardi E, Merlino G, Welker P, Handjiski B, Roloff B, Paus R. Involvement of hepatocyte growth factor/scatter factor and met receptor signaling in hair follicle morphogenesis and cycling. FASEB J. 2000;14:319–32.
39. Paus R, Maurer M, Slominski A, Czarnetzki BM. Mast cell involvement in murine hair growth. Dev Biol. 1994;163:230–40.
40. Philp D, Nguyen M, Scheremeta B, St-Surin S, Villa AM, Orgel A, Kleinman HK, Elkin M. Thymosin beta4 increases hair growth by activation of hair follicle stem cells. FASEB J. 2004;18:385–7.
41. Muller-Rover S, Peters EJ, Botchkarev VA, Panteleyev A, Paus R. Distinct patterns of NCAM expression are associated with defined stages of murine hair follicle morphogenesis and regression. J Histochem Cytochem. 1998;46:1401–10.
42. Wilson C, Cotsarelis G, Wei ZG, Fryer E, Margolis-Fryer J, Ostead M, Tokarek R, Sun TT, Lavker RM. Cells within the bulge region of mouse hair follicle transiently proliferate during

early anagen: heterogeneity and functional differences of various hair cycles. Differentiation. 1994;55:127–36.
43. Muller-Rover S, Bulfone-Paus S, Handjiski B, Welker P, Sundberg JP, McKay IA, Botchkarev VA, Paus R. Intercellular adhesion molecule-1 and hair follicle regression. J Histochem Cytochem. 2000;48:557–68.
44. Sato N, Leopold PL, Crystal RG. Induction of the hair growth phase in postnatal mice by localized transient expression of Sonic hedgehog. J Clin Investig. 1999;104:855–64.
45. Zhou P, Byrne C, Jacobs J, Fuchs E. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes Dev. 1995;9:700–13.
46. DasGupta R, Fuchs E. Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development. 1999;126:4557–68.
47. Tsuboi R, Yamazaki M, Matsuda Y, Uchida K, Ueki R, Ogawa H. Antisense oligonucleotide targeting fibroblast growth factor receptor (FGFR)-1 stimulates cellular activity of hair follicles in an in vitro organ culture system. Int J Dermatol. 2007;46:259–63.
48. Mak KK, Chan SY. Epidermal growth factor as a biologic switch in hair growth cycle. J Biol Chem. 2003;278:26120–6.
49. Weger N, Schlake T. Igf-I signalling controls the hair growth cycle and the differentiation of hair shafts. J Investig Dermatol. 2005;125:873–82.
50. Sowden HM, Karoo RO, Tobin DJ. Transforming growth factor-beta receptor II is preferentially expressed in the companion layer of the human anagen hair follicle. Br J Dermatol. 2007;157:161–4.
51. Darnell JE Jr. STATs and gene regulation. Science. 1997;277:1630–5.
52. Sano S, Kira M, Takagi S, Yoshikawa K, Takeda J, Itami S. Two distinct signaling pathways in hair cycle induction: Stat3-dependent and -independent pathways. Proc Natl Acad Sci U S A. 2000;97:13824–9.
53. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the epithelial stem cell niche in skin. Science. 2004;303:359–63.
54. Mackenzie IC. Retroviral transduction of murine epidermal stem cells demonstrates clonal units of epidermal structure. J Investig Dermatol. 1997;109:377–83.
55. Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature. 2007;447:316–20.
56. Kaur P. Interfollicular epidermal stem cells: identification, challenges, potential. J Investig Dermatol. 2006;126:1450–8.
57. Jones PH, Watt FM. Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. Cell. 1993;73:713–24.
58. Horsley V, O'Carroll D, Tooze R, Ohinata Y, Saitou M, Obukhanych T, Nussenzweig M, Tarakhovsky A, Fuchs E. Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. Cell. 2006;126:597–609.
59. Jensen UB, Yan X, Triel C, Woo SH, Christensen R, Owens DM. A distinct population of clonogenic and multipotent murine follicular keratinocytes residing in the upper isthmus. J Cell Sci. 2008;121:609–17.
60. Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G. Capturing and profiling adult hair follicle stem cells. Nat Biotechnol. 2004;22:411–7.
61. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med. 2005;11:1351–4.
62. Silva-Vargas V, Lo Celso C, Giangreco A, Ofstad T, Prowse DM, Braun KM, Watt FM. Beta-catenin and Hedgehog signal strength can specify number and location of hair follicles in adult epidermis without recruitment of bulge stem cells. Dev Cell. 2005;9:121–31.
63. Nguyen H, Merrill BJ, Polak L, Nikolova M, Rendl M, Shaver TM, Pasolli HA, Fuchs E. Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. Nat Genet. 2009;41:1068–75.
64. Blanpain C, Fuchs E. Stem cell plasticity. Plasticity of epithelial stem cells in tissue regeneration. Science. 2014;344:1242281.

65. Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niche. Cell. 2004;116:769–78.
66. Cotsarelis G. Epithelial stem cells: a folliculocentric view. J Investig Dermatol. 2006;126:1459–68.
67. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, Reece JM, Tennant RW. Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. J Investig Dermatol. 2003;120:501–11.
68. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell. 2004;118:635–48.
69. Tani H, Morris RJ, Kaur P. Enrichment for murine keratinocyte stem cells based on cell surface phenotype. Proc Natl Acad Sci U S A. 2000;97:10960–5.
70. Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet. 2008;40:1291–9.
71. Levy V, Lindon C, Harfe BD, Morgan BA. Distinct stem cell populations regenerate the follicle and interfollicular epidermis. Dev Cell. 2005;9:855–61.
72. Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, Dela Cruz-Racelis J, Fuchs E. A two-step mechanism for stem cell activation during hair regeneration. Cell Stem Cell. 2009;4:155–69.
73. Kobielak K, Stokes N, de la Cruz J, Polak L, Fuchs E. Loss of a quiescent niche but not follicle stem cells in the absence of bone morphogenetic protein signaling. Proc Natl Acad Sci U S A. 2007;104:10063–8.
74. Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. Genes Dev. 2005;19:1596–611.
75. Levy V, Lindon C, Zheng Y, Harfe BD, Morgan BA. Epidermal stem cells arise from the hair follicle after wounding. FASEB J. 2007;21:1358–66.
76. Nowak JA, Polak L, Pasolli HA, Fuchs E. Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell. 2008;3:33–43.
77. Petersson M, Brylka H, Kraus A, John S, Rappl G, Schettina P, Niemann C. TCF/Lef1 activity controls establishment of diverse stem and progenitor cell compartments in mouse epidermis. EMBO J. 2011;30:3004–18.
78. Nijhof JG, Braun KM, Giangreco A, van Pelt C, Kawamoto H, Boyd RL, Willemze R, Mullenders LH, Watt FM, de Gruijl FR, van Ewijk W. The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristics of progenitor cells. Development. 2006;133:3027–37.
79. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, Stange DE, Toftgard R, Clevers H. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science. 2010;327:1385–9.
80. Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, Itami S, Watt FM. Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell. 2009;4:427–39.
81. Brownell I, Guevara E, Bai CB, Loomis CA, Joyner AL. Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells. Cell Stem Cell. 2011;8:552–65.
82. Van Keymeulen A, Rocha AS, Ousset M, Beck B, Bouvencourt G, Rock J, Sharma N, Dekoninck S, Blanpain C. Distinct stem cells contribute to mammary gland development and maintenance. Nature. 2011;479:189–93.
83. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE. Generation of a functional mammary gland from a single stem cell. Nature. 2006;439:84–8.
84. Biedermann T, Pontiggia L, Bottcher-Haberzeth S, Tharakan S, Braziulis E, Schiestl C, Meuli M, Reichmann E. Human eccrine sweat gland cells can reconstitute a stratified epidermis. J Investig Dermatol. 2010;130:1996–2009.
85. Fu XB, Sun TZ, Li XK, Sheng ZY. Morphological and distribution characteristics of sweat glands in hypertrophic scar and their possible effects on sweat gland regeneration. Chin Med J. 2005;118:186–91.

86. Schon M, Benwood J, O'Connell-Willstaedt T, Rheinwald JG. Human sweat gland myoepithelial cells express a unique set of cytokeratins and reveal the potential for alternative epithelial and mesenchymal differentiation states in culture. J Cell Sci. 1999;112(Pt 12):1925–36.
87. Xie J, Yao B, Han Y, Shang T, Gao D, Yang S, Ma K, Huang S, Fu X. Cytokeratin expression at different stages in sweat gland development of C57BL/6J Mice. Int J Low Extrem Wounds. 2015;14:365–71.
88. Xie J, Yao B, Han Y, Huang S, Fu X. Skin appendage-derived stem cells: cell biology and potential for wound repair. Burns Trauma. 2016;4:38.
89. Cottle DL, Kretzschmar K, Schweiger PJ, Quist SR, Gollnick HP, Natsuga K, Aoyagi S, Watt FM. c-MYC-induced sebaceous gland differentiation is controlled by an androgen receptor/p53 axis. Cell Rep. 2013;3:427–41.
90. Page ME, Lombard P, Ng F, Gottgens B, Jensen KB. The epidermis comprises autonomous compartments maintained by distinct stem cell populations. Cell Stem Cell. 2013;13:471–82.
91. Chunmeng S, Tianmin C. Skin: a promising reservoir for adult stem cell populations. Med Hypotheses. 2004;62:683–8.
92. Myers TJ, Granero-Molto F, Longobardi L, Li T, Yan Y, Spagnoli A. Mesenchymal stem cells at the intersection of cell and gene therapy. Expert Opin Biol Ther. 2010;10:1663–79.
93. Zhang J, Huang X, Wang H, Liu X, Zhang T, Wang Y, Hu D. The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy. Stem Cell Res Ther. 2015;6:234.
94. Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, Shrayer D, Carson P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng. 2007;13:1299–312.
95. Vojtassak J, Danisovic L, Kubes M, Bakos D, Jarabek L, Ulicna M, Blasko M. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. Neuro Endocrinol Lett. 2006;27(Suppl 2):134–7.
96. de la Garza-Rodea AS, Knaan-Shanzer S, van Bekkum DW. Pressure ulcers: description of a new model and use of mesenchymal stem cells for repair. Dermatology. 2011;223:266–84.
97. Gardien KL, Middelkoop E, Ulrich MM. Progress towards cell-based burn wound treatments. Regen Med. 2014;9:201–18.
98. Liu L, Yu Y, Hou Y, Chai J, Duan H, Chu W, Zhang H, Hu Q, Du J. Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PLoS One. 2014;9:e88348.
99. Stoff A, Rivera AA, Sanjib Banerjee N, Moore ST, Michael Numnum T, Espinosa-de-Los-Monteros A, Richter DF, Siegal GP, Chow LT, Feldman D, Vasconez LO, Michael Mathis J, Stoff-Khalili MA, Curiel DT. Promotion of incisional wound repair by human mesenchymal stem cell transplantation. Exp Dermatol. 2009;18:362–9.
100. Bura A, Planat-Benard V, Bourin P, Silvestre JS, Gross F, Grolleau JL, Saint-Lebese B, Peyrafitte JA, Fleury S, Gadelorge M, Taurand M, Dupuis-Coronas S, Leobon B, Casteilla L. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. Cytotherapy. 2014;16:245–57.
101. Nuschke A. Activity of mesenchymal stem cells in therapies for chronic skin wound healing. Organogenesis. 2014;10:29–37.
102. Paunescu V, Deak E, Herman D, Siska IR, Tanasie G, Bunu C, Anghel S, Tatu CA, Oprea TI, Henschler R, Ruster B, Bistrian R, Seifried E. In vitro differentiation of human mesenchymal stem cells to epithelial lineage. J Cell Mol Med. 2007;11:502–8.
103. Tang QQ, Lane MD. Adipogenesis: from stem cell to adipocyte. Annu Rev Biochem. 2012;81:715–36.
104. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells. 2004;22:377–84.
105. Wu Y, Huang S, Enhe J, Ma K, Yang S, Sun T, Fu X. Bone marrow-derived mesenchymal stem cell attenuates skin fibrosis development in mice. Int Wound J. 2014;11:701–10.
106. Ward CL, Sanchez CJ Jr, Pollot BE, Romano DR, Hardy SK, Becerra SC, Rathbone CR, Wenke JC. Soluble factors from biofilms of wound pathogens modulate human bone

marrow-derived stromal cell differentiation, migration, angiogenesis, and cytokine secretion. BMC Microbiol. 2015;15:75.
107. Wu Y, Zhao RC, Tredget EE. Concise review: bone marrow-derived stem/progenitor cells in cutaneous repair and regeneration. Stem Cells. 2010;28:905–15.
108. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev. 2015;24:1635–47.
109. Mebarki M, Coquelin L, Layrolle P, Battaglia S, Tossou M, Hernigou P, Rouard H, Chevallier N. Enhanced human bone marrow mesenchymal stromal cell adhesion on scaffolds promotes cell survival and bone formation. Acta Biomater. 2017;59:94–107.
110. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci. 2007;48:15–24.
111. Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, Park BS, Sung JH. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. Wound Repair Regen. 2009;17:540–7.
112. Wettstein R, Savic M, Pierer G, Scheufler O, Haug M, Halter J, Gratwohl A, Baumberger M, Schaefer DJ, Kalbermatten DF. Progenitor cell therapy for sacral pressure sore: a pilot study with a novel human chronic wound model. Stem Cell Res Ther. 2014;5:18.
113. Fu X, Sun X. Can hematopoietic stem cells be an alternative source for skin regeneration? Ageing Res Rev. 2009;8:244–9.
114. Kirby GT, Mills SJ, Cowin AJ, Smith LE. Stem cells for cutaneous wound healing. Biomed Res Int. 2015;2015:285869.
115. Kim JY, Suh W. Stem cell therapy for dermal wound healing. Int J Stem Cells. 2010;3:29–31.
116. Suh W, Kim KL, Kim JM, Shin IS, Lee YS, Lee JY, Jang HS, Lee JS, Byun J, Choi JH, Jeon ES, Kim DK. Transplantation of endothelial progenitor cells accelerates dermal wound healing with increased recruitment of monocytes/macrophages and neovascularization. Stem Cells. 2005;23:1571–8.
117. Kim KL, Han DK, Park K, Song SH, Kim JY, Kim JM, Ki HY, Yie SW, Roh CR, Jeon ES, Kim DK, Suh W. Enhanced dermal wound neovascularization by targeted delivery of endothelial progenitor cells using an RGD-g-PLLA scaffold. Biomaterials. 2009;30:3742–8.
118. Di Santo S, Yang Z, Wyler von Ballmoos M, Voelzmann J, Diehm N, Baumgartner I, Kalka C. Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation. PLoS One. 2009;4:e5643.
119. Fernandes KJ, McKenzie IA, Mill P, Smith KM, Akhavan M, Barnabe-Heider F, Biernaskie J, Junek A, Kobayashi NR, Toma JG, Kaplan DR, Labosky PA, Rafuse V, Hui CC, Miller FD. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol. 2004;6:1082–93.
120. Fernandes KJ, Toma JG, Miller FD. Multipotent skin-derived precursors: adult neural crest-related precursors with therapeutic potential. Philos Trans R Soc Lond Ser B Biol Sci. 2008;363:185–98.
121. Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G, Herlyn M, Xu X. Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol. 2006;168:1879–88.
122. Uchugonova A, Duong J, Zhang N, Konig K, Hoffman RM. The bulge area is the origin of nestin-expressing pluripotent stem cells of the hair follicle. J Cell Biochem. 2011;112:2046–50.
123. Sun X, Fu X, Han W, Zhao M, Chalmers L. Epidermal stem cells: an update on their potential in regenerative medicine. Expert Opin Biol Ther. 2013;13:901–10.
124. Mii S, Duong J, Tome Y, Uchugonova A, Liu F, Amoh Y, Saito N, Katsuoka K, Hoffman RM. Nestin-expressing Hair-Follicle-Associated Pluripotent (HAP) stem cells promote whisker sensory-nerve growth in long-term 3D-Gelfoam(R) histoculture. Methods Mol Biol. 2016;1453:39–47.

125. Amoh Y, Li L, Katsuoka K, Penman S, Hoffman RM. Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons. Proc Natl Acad Sci U S A. 2005;102:5530–4.
126. Amoh Y, Katsuoka K, Hoffman RM. The advantages of hair follicle pluripotent stem cells over embryonic stem cells and induced pluripotent stem cells for regenerative medicine. J Dermatol Sci. 2010;60:131–7.
127. Clark RA, Yamanaka K, Bai M, Dowgiert R, Kupper TS. Human skin cells support thymus-independent T cell development. J Clin Investig. 2005;115:3239–49.
128. Liang L, Bickenbach JR. Somatic epidermal stem cells can produce multiple cell lineages during development. Stem Cells. 2002;20:21–31.
129. Li J, Greco V, Guasch G, Fuchs E, Mombaerts P. Mice cloned from skin cells. Proc Natl Acad Sci U S A. 2007;104:2738–43.
130. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilic J, Pekarik V, Tiscornia G, Edel M, Boue S, Izpisua Belmonte JC. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol. 2008;26:1276–84.
131. Bull JJ, Muller-Rover S, Patel SV, Chronnell CM, McKay IA, Philpott MP. Contrasting localization of c-Myc with other Myc superfamily transcription factors in the human hair follicle and during the hair growth cycle. J Investig Dermatol. 2001;116:617–22.
132. Barajon I, Rumio C, Donetti E, Imberti A, Brivio M, Castano P. Pattern of expression of c-Myc, Max and Bin1 in human anagen hair follicles. Br J Dermatol. 2001;144:1193–203.
133. Gandarillas A, Watt FM. c-Myc promotes differentiation of human epidermal stem cells. Genes Dev. 1997;11:2869–82.
134. Gebhardt A, Frye M, Herold S, Benitah SA, Braun K, Samans B, Watt FM, Elsasser HP, Eilers M. Myc regulates keratinocyte adhesion and differentiation via complex formation with Miz1. J Cell Biol. 2006;172:139–49.
135. Segre JA, Bauer C, Fuchs E. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet. 1999;22:356–60.
136. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T, Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science. 2008;321:699–702.
137. Tsai SY, Clavel C, Kim S, Ang YS, Grisanti L, Lee DF, Kelley K, Rendl M. Oct4 and klf4 reprogram dermal papilla cells into induced pluripotent stem cells. Stem Cells. 2010;28:221–8.
138. Braun KM, Niemann C, Jensen UB, Sundberg JP, Silva-Vargas V, Watt FM. Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development. 2003;130:5241–55.
139. Rompolas P, Mesa KR, Greco V. Spatial organization within a niche as a determinant of stem-cell fate. Nature. 2013;502:513–8.
140. Schepeler T, Page ME, Jensen KB. Heterogeneity and plasticity of epidermal stem cells. Development. 2014;141:2559–67.
141. Ge Y, Fuchs E. Stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. Nat Rev Genet. 2018;19(5):311.
142. Lu CP, Polak L, Rocha AS, Pasolli HA, Chen SC, Sharma N, Blanpain C, Fuchs E. Identification of stem cell populations in sweat glands and ducts reveals roles in homeostasis and wound repair. Cell. 2012;150:136–50.
143. Kasper M, Jaks V, Are A, Bergstrom A, Schwager A, Svard J, Teglund S, Barker N, Toftgard R. Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. Proc Natl Acad Sci U S A. 2011;108:4099–104.
144. Youssef KK, Van Keymeulen A, Lapouge G, Beck B, Michaux C, Achouri Y, Sotiropoulou PA, Blanpain C. Identification of the cell lineage at the origin of basal cell carcinoma. Nat Cell Biol. 2010;12:299–305.
145. Wong SY, Reiter JF. Wounding mobilizes hair follicle stem cells to form tumors. Proc Natl Acad Sci U S A. 2011;108:4093–8.

146. Vidal VP, Chaboissier MC, Lutzkendorf S, Cotsarelis G, Mill P, Hui CC, Ortonne N, Ortonne JP, Schedl A. Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. Curr Biol. 2005;15:1340–51.
147. Vidal VP, Ortonne N, Schedl A. SOX9 expression is a general marker of basal cell carcinoma and adnexal-related neoplasms. J Cutan Pathol. 2008;35:373–9.
148. Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR, Camargo FD. Yap1 acts downstream of alpha-catenin to control epidermal proliferation. Cell. 2011;144:782–95.
149. Takeda H, Lyle S, Lazar AJ, Zouboulis CC, Smyth I, Watt FM. Human sebaceous tumors harbor inactivating mutations in LEF1. Nat Med. 2006;12:395–7.
150. Lo Celso C, Prowse DM, Watt FM. Transient activation of beta-catenin signalling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours. Development. 2004;131:1787–99.
151. Niemann C, Owens DM, Schettina P, Watt FM. Dual role of inactivating Lef1 mutations in epidermis: tumor promotion and specification of tumor type. Cancer Res. 2007;67:2916–21.
152. Malanchi I, Peinado H, Kassen D, Hussenet T, Metzger D, Chambon P, Huber M, Hohl D, Cano A, Birchmeier W, Huelsken J. Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. Nature. 2008;452:650–3.
153. Arwert EN, Hoste E, Watt FM. Epithelial stem cells, wound healing and cancer. Nat Rev Cancer. 2012;12:170–80.
154. Baker CM, Verstuyf A, Jensen KB, Watt FM. Differential sensitivity of epidermal cell subpopulations to beta-catenin-induced ectopic hair follicle formation. Dev Biol. 2010;343:40–50.
155. Hutchin ME, Kariapper MS, Grachtchouk M, Wang A, Wei L, Cummings D, Liu J, Michael LE, Glick A, Dlugosz AA. Sustained Hedgehog signaling is required for basal cell carcinoma proliferation and survival: conditional skin tumorigenesis recapitulates the hair growth cycle. Genes Dev. 2005;19:214–23.
156. Dahmane N, Lee J, Robins P, Heller P, Ruiz i Altaba A. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. Nature. 1997;389:876–81.
157. Nilsson M, Unden AB, Krause D, Malmqwist U, Raza K, Zaphiropoulos PG, Toftgard R. Induction of basal cell carcinomas and trichoepitheliomas in mice overexpressing GLI-1. Proc Natl Acad Sci U S A. 2000;97:3438–43.
158. Sheng H, Goich S, Wang A, Grachtchouk M, Lowe L, Mo R, Lin K, de Sauvage FJ, Sasaki H, Hui CC, Dlugosz AA. Dissecting the oncogenic potential of Gli2: deletion of an NH(2)-terminal fragment alters skin tumor phenotype. Cancer Res. 2002;62:5308–16.
159. Wang GY, Wang J, Mancianti ML, Epstein EH Jr. Basal cell carcinomas arise from hair follicle stem cells in Ptch1(+/-) mice. Cancer Cell. 2011;19:114–24.
160. Matic M. A subpopulation of human basal keratinocytes has a low/negative MHC class I expression. Hum Immunol. 2005;66:962–8.
161. Christoph T, Muller-Rover S, Audring H, Tobin DJ, Hermes B, Cotsarelis G, Ruckert R, Paus R. The human hair follicle immune system: cellular composition and immune privilege. Br J Dermatol. 2000;142:862–73.
162. Ojeh N, Pastar I, Tomic-Canic M, Stojadinovic O. Stem cells in skin regeneration, wound healing, and their clinical applications. Int J Mol Sci. 2015;16:25476–501.
163. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.
164. Hanna J, Markoulaki S, Schorderet P, Carey BW, Beard C, Wernig M, Creyghton MP, Steine EJ, Cassady JP, Foreman R, Lengner CJ, Dausman JA, Jaenisch R. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell. 2008;133:250–64.
165. Itoh M, Kiuru M, Cairo MS, Christiano AM. Generation of keratinocytes from normal and recessive dystrophic epidermolysis bullosa-induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2011;108:8797–802.
166. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature. 2008;455:627–32.

167. Forsberg M, Carlen M, Meletis K, Yeung MS, Barnabe-Heider F, Persson MA, Aarum J, Frisen J. Efficient reprogramming of adult neural stem cells to monocytes by ectopic expression of a single gene. Proc Natl Acad Sci U S A. 2010;107:14657–61.
168. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010;142:375–86.
169. Takeuchi JK, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature. 2009;459:708–11.
170. Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, Russo G, Carninci P, Pezzoli G, Gainetdinov RR, Gustincich S, Dityatev A, Broccoli V. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature. 2011;476:224–7.
171. Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M. Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci U S A. 2011;108:10343–8.
172. Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? Nat Biotechnol. 2011;29:892–907.
173. Davidson EH. Emerging properties of animal gene regulatory networks. Nature. 2010;468:911–20.
174. Mauda-Havakuk M, Litichever N, Chernichovski E, Nakar O, Winkler E, Mazkereth R, Orenstein A, Bar-Meir E, Ravassard P, Meivar-Levy I, Ferber S. Ectopic PDX-1 expression directly reprograms human keratinocytes along pancreatic insulin-producing cells fate. PLoS One. 2011;6:e26298.