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EDITED BY

Shiyu Xia,
California Institute of Technology,
United States

REVIEWED BY

Le Xiao,
Boston Children's Hospital and Harvard
Medical School, United States
Ahmet Bugra Tufan,
Harvard Medical School, United States

*CORRESPONDENCE

Yangtuo Luo
✉ ytluo@cmu.edu.cn
Xiaochun Ma
✉ maxc_cmu@sina.com

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Role of pyroptosis in hemostasis activation in sepsis

Chengrui Zhu¹, Yingjian Liang¹, Yangtuo Luo^{2*}
and Xiaochun Ma^{1*}

¹Department of Critical Care Medicine, The First Hospital of China Medical University, Shenyang, Liaoning, China, ²Department of Otolaryngology, The First Hospital of China Medical University, Shenyang, Liaoning, China

Sepsis is frequently associated with hemostasis activation and thrombus formation, and systematic hemostatic changes are associated with a higher risk of mortality. The key events underlying hemostasis activation during sepsis are the strong activation of innate immune pathways and the excessive inflammatory response triggered by invading pathogens. Pyroptosis is a proinflammatory form of programmed cell death, that defends against pathogens during sepsis. However, excessive pyroptosis can lead to a dysregulation of host immune responses and organ dysfunction. Recently, pyroptosis has been demonstrated to play a prominent role in hemostasis activation in sepsis. Several studies have demonstrated that pyroptosis participates in the release and coagulation activity of tissue factors. In addition, pyroptosis activates leukocytes, endothelial cells, platelets, which cooperate with the coagulation cascade, leading to hemostasis activation in sepsis. This review article attempts to interpret the molecular and cellular mechanisms of the hemostatic imbalance induced by pyroptosis during sepsis and discusses potential therapeutic strategies.

KEYWORDS

pyroptosis, inflammasome, coagulation, hemostasis, sepsis, tissue factor

1 Introduction

Sepsis is defined as a life-threatening condition caused by a dysregulated host response to infection (1). It has been recognized as a global health priority by the World Health Organization and is the most common cause of death in hospitals, causing 11 million deaths worldwide per year (2). During sepsis, the invading pathogen encounters the host innate immune system triggering a sustained systemic inflammatory response and coagulation cascade. The co-dependency of innate immune pathways and the hemostasis process is termed “immunothrombosis” or “immunocoagulation” (3). The “immunothrombosis” process serves as the non-specific first line of host defense against pathogens. However, the over-activation of the hemostatic system leads to excessive thrombin formation and hyperfibrinolysis, resulting in disseminated intravascular coagulation (DIC) and ultimately to multiple organ dysfunction and septic death (4).

Pyroptosis, a recently discovered programmed mode of cell death, participates in the innate immune response, inhibiting the replication of intracellular pathogens, and activating

immune cells to phagocytose and kill pathogens (5, 6). However, overactivated pyroptosis leads to dysregulated host immune response and organ dysfunction (7, 8). Pyroptosis occurs in many cell types and participates in the pathophysiological processes of sepsis (9). More recently, pyroptosis has been shown to play a prominent role in the prothrombotic response in sepsis (10, 11). This review article attempts to interpret the molecular and cellular mechanisms of the hemostatic imbalance induced by pyroptosis in sepsis and discusses potential therapeutic strategies.

2 Process of hemostasis and thrombosis

Hemostasis is an important physiological process that prevents bleeding after a blood vessel injury and involves two main mechanisms: blood coagulation and platelet activation. Thrombosis is generally considered to be the pathological progression of hemostasis, and refers to thrombus formation inside blood vessels, resulting in the occlusion of arteries, veins, and microvessels (12). It is a critical event in many diseases, including myocardial infarction, stroke, venous thromboembolism, and DIC. Hemostasis and thrombosis share the core pathways of platelet activation and the coagulation cascade, and the processes will be briefly described to better understand the underlying mechanisms of hemostasis imbalance in sepsis (Figure 1).

When blood vessels are disrupted, collagen in the subendothelial matrix and tissue factor (TF) are exposed to the blood, which initiates thrombus formation (12–14). Platelets are recruited to the injury sites through the efficient interaction of cell-surface receptors with the

exposed collagen and released von Willebrand factor (vWF) in the subendothelial matrix (15), then the platelets become activated. In addition, exposed TF also initiates platelet activation by generating thrombin through the coagulation cascade (16). Thrombin cleaves protease-activated receptor 4 in platelets and activates the platelets (17). After the recruitment and activation stages, platelets firmly adhere to the vessel wall and promote continued platelet activation and aggregation, resulting in rapid thrombus growth (12).

In parallel with platelet recruitment, the coagulation cascade is initiated to stabilize and reinforce the platelet thrombus (12). The coagulation cascade, first described in 1964, involves the sequential activation of a series of plasma serine proteases in the generation of activated thrombin (18, 19). The classical view of the “cascade” model distinguishes two initiation pathways: the extrinsic pathway and the intrinsic pathway. The extrinsic pathway is initiated by TF (also known as factor III), which is expressed by vessel-wall cells that are not exposed to blood and by tissue cells (20). With TF exposure to blood, it binds to factor VIIa and triggers the coagulation cascade (13). The TF-initiated pathway is activated by trauma, infection, or the disruption of the vessel wall under normal hemostasis (13, 21). The intrinsic pathway of coagulation, also known as the contact pathway, begins upon exposure of the “contact” factor (factor XII) in plasma to a negatively charged surface (18). The exposed endothelial cells, platelets, collagen, and foreign surfaces, as acting surfaces, initiate factor XII activation and trigger a chain reaction of coagulation factors (18). However, this pathway is not linked to normal hemostasis, and instead contributes predominantly to pathological thrombosis (22). When coagulation factor X is activated through the intrinsic or extrinsic pathways, it converts prothrombin to thrombin (19). Thrombin further cleaves

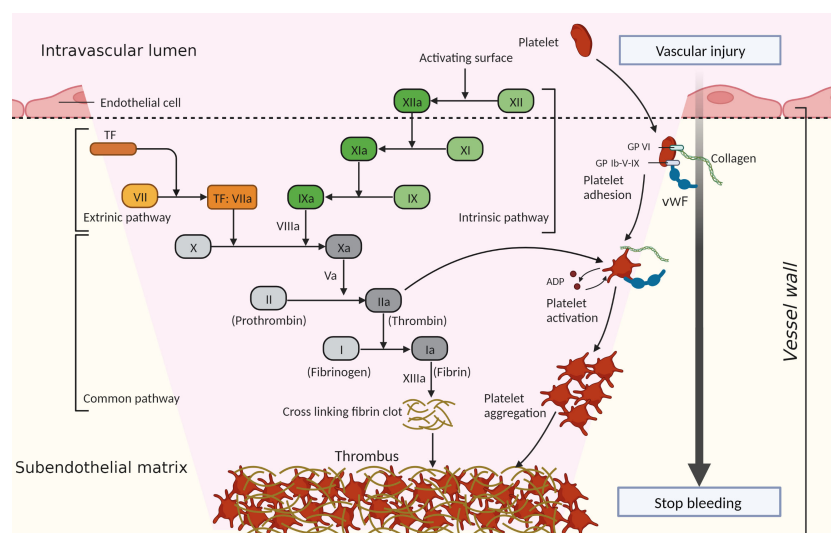


FIGURE 1

Process of hemostasis and thrombosis. Hemostasis and thrombosis share the core pathways of platelet activation and the coagulation cascade, which act together to generate a hemostatic clot or thrombus. Platelets are recruited to a site of vessel injury where collagen is exposed through the interaction of cell-surface receptors (such as glycoprotein VI [GP VI], glycoprotein Ib-V-IX [GP Ib-V-IX], and some integrins [not shown]) with the collagen and released von Willebrand factor (vWF). Then platelets are activated and adhere to the vessel wall, releasing agonists (such as adenosine diphosphate [ADP]), which promote continued platelet activation and aggregation. The extrinsic pathway is initiated by exposed tissue factor (TF, also known as factor III). TF forms a complex with circulating factor VIIa, thereby triggering the coagulation cascade. The intrinsic pathway begins with the contact of factor XII with the negatively charged surface. Factor XII is activated and triggers a chain reaction of coagulation factors. When coagulation factor X is activated through the intrinsic or extrinsic pathways, it converts prothrombin (factor II) to thrombin (factor IIa). Thrombin further cleaves fibrinogen (factor I) into fibrin (factor Ia), forming a mesh that binds to and strengthens the platelet clot and stops bleeding.

fibrinogen into fibrin, forming a mesh that binds to and strengthens the platelet clot and stops bleeding. This is known as the common pathway (18, 19, 22).

During hemostasis and thrombosis, platelet thrombus formation and fibrin deposition occur concomitantly and are connected with each other (3). Under normal conditions, regulatory mechanisms of anticoagulation (such as tissue factor pathway inhibitor [TFPI], protein C system, antithrombin [AT]) and fibrinolysis systems restrict blood clot formation in a temporary and localized manner (23). However, when pathological processes overwhelm the regulatory mechanisms of hemostasis, thrombosis is initiated (12), occurring as arterial and venous thrombosis or DIC.

3 Hemostasis activation in sepsis

During sepsis, pathogens trigger an impaired host response, leading to innate immune response, systemic inflammation, and eventually activation of the coagulation response (24, 25). Clinically, almost all patients with sepsis have hemostasis abnormalities, ranging from slight coagulation marker changes to stronger coagulation activation with a decrease in platelet counts and prolongation of coagulation time, to fulminant coagulation activation with widespread micro-thrombosis and profuse bleeding, known as DIC (26).

In general, blood cells, that is, monocytes, neutrophils, platelets, and vascular endothelial cells, together with coagulation factors, play significant roles in hemostatic changes in sepsis (24, 27). Moreover, in addition to increased coagulation, the hemostatic change also involves the downregulation of anticoagulant mechanisms and an impaired fibrinolytic response (24). Importantly, the key events underlying the hemostasis activation in sepsis are the strong activation of innate immune cells and an excessive inflammatory response (23). “Immunothrombosis” has been therefore introduced to emphasize the pivotal role of the innate immune response in hemostasis activation and thrombosis, which can protect against pathogens in the vascular system and are also involved in pathological thrombosis (3). Multiple immune-related molecular and cellular factors contribute to hemostasis activation in sepsis, which is generally supported by distinct cells and molecules that are irrelevant to physiological hemostasis. The roles of innate immune cells and immune-related factors in hemostasis activation in sepsis are summarized below (Figure 2).

3.1 Tissue factor

The most principal initiator of coagulation activation in sepsis is TF (24). TF is a transmembrane protein constitutively expressed by vessel

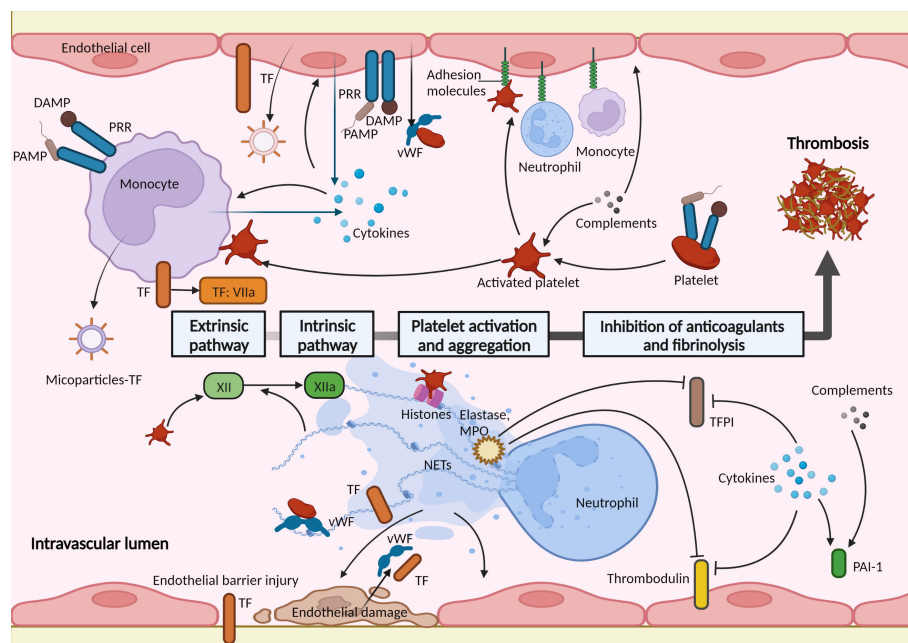


FIGURE 2

Immune-related factors in hemostasis activation during sepsis. Activation of the coagulation pathway, platelet activation and aggregation, and impaired anticoagulant and fibrinolytic pathways act cumulatively to a procoagulant state in sepsis. These are mediated by multiple immune-related molecular and cellular factors. Monocytes and endothelial cells play important roles in thrombus formation during sepsis. They express and release tissue factor (TF) when recognizing pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs). The activated endothelial cells serve as a bridge between the coagulation response and immune factors. Endothelial cells expressed adhesion molecules (such as E-selectin and intercellular adhesion molecule 1) mediating the adhesion and interactions of immune cells. The injured endothelial barrier loses antithrombotic function and induces exposure of TF; the damaged endothelial cells release prothrombotic molecules, such as von Willebrand factor (vWF) and TF. Platelets are activated and interact with circulating leukocytes and endothelial cells, and trigger factor XII initiating the intrinsic coagulation pathway. Neutrophil extracellular traps (NETs), released by neutrophils, support immunothrombosis in several ways: activate and damage endothelial cells; NETs and histones in NETs promote the recruitment and activation of platelets; bind to TF and activate factor XII; neutrophil elastase and myeloperoxidase (MPO) in NETs inhibit the anticoagulants. Activated complements activate endothelial cells and platelets and increase the level of plasminogen activator inhibitor-1 (PAI-1). Proinflammatory cytokines increase the expression of TF in monocytes and endothelial cells; activate leukocytes, platelets, and endothelial cells; downregulate anticoagulants and fibrinolysis pathways. TFPI, tissue factor pathway inhibitor.

wall cells and can also be expressed in circulating blood cells induced by stimulus (21, 28). During sepsis, invading pathogens encounter the innate immune system, and immune cells recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs) (29). In response to this, activated monocytes and endothelial cells, as well as their microparticles, express and release activated TF at pathogen exposure sites (30, 31). Moreover, the endothelial barrier injury during sepsis leads to TF exposure to coagulation factors in the blood (32). Cumulatively, TF initiates the extrinsic coagulation pathway and mediates the coagulation response in sepsis (24).

3.2 Neutrophil extracellular traps

Neutrophil extracellular traps (NETs) are DNA- and histone-based, web-like structures released by neutrophils to help capture pathogens (33). NETs promote inflammation and tissue damage during sepsis and support immunothrombosis in several ways (34). First, NETs can damage and activate endothelial cells (35). Due to their inherent ability to kill pathogens, the structures of NETs are extremely cytotoxic to host cells, damaging and killing endothelial cells, and leading to coagulation activation (36). NETs activate endothelial cells by inducing them to release adhering molecules and TF, and subsequently recruiting inflammatory cells and promoting inflammation and coagulation (35). Second, NETs and histones in NETs bind to vWF and promote the recruitment and activation of platelets (37–39). Third, NETs can promote the extrinsic pathway of coagulation by binding to TF, and the polyanionic surface of NETs activates the contact activation protein, factor XII, thereby activating the intrinsic pathway (40). Finally, neutrophil elastase and myeloperoxidase in NETs upregulate the procoagulant response through proteolytic cleavage and the oxidation of anticoagulants, such as thrombomodulin and TFPI (41, 42).

3.3 Platelets

Platelets are regarded as major actors in physiological hemostasis and are front-runners during sepsis (43). Currently, platelets are regarded as immune cells. During sepsis, platelets can be recruited and activated in a process closely resembling physiological hemostasis (44). Furthermore, when stimulated by PAMPs, DAMPs, and immune cells, platelets are activated by different platelet receptors such as glycoprotein Ib-V-IX, integrin α Ib β 3 (GPIIb/IIIa), and toll-like receptors (TLRs) (45). Once activated, platelets express adhesion receptors (for example, P-selection and CD 40 ligand) and release chemokines that recruit and interact with circulating leukocytes (43), amplifying “immunothrombosis”. In addition, platelet-derived polyphosphates activate factor XII, thereby initiating the intrinsic coagulation pathway (46).

3.4 Endothelial cells

The integrity of the endothelial barrier structure and function allows for antithrombotic function. The barrier function is maintained by the

endothelial cytoskeleton, glycocalyx, intercellular adhesion molecules, and other proteins (47). During sepsis, endothelial activation and dysfunction are key events, that act as a bridge between the immune response and the coagulation cascade (47). Circulating PAMPs, DAMPs, and proinflammatory cytokines activate endothelial cells, which leads to the increased expression and release of adhesion molecules, mediating the adhesion and interactions of activated leukocytes and platelets (27, 48). Furthermore, injured or activated endothelial cells assume hypercoagulability with the release or expression of prothrombotic components, such as vWF and TF, and impairment of the membrane anticoagulant components, such as TFPI, thrombomodulin, and endothelial glycocalyx (27, 32).

3.5 Complements and cytokines

The complement system consists of numerous plasma and membrane-bound proteins, and functions as an intravascular surveillance system by killing pathogens (49). It is an essential component of the innate and adaptive immune systems (49). Complement activation, including C3a and C5a, also promotes coagulation and mediates the activation of endothelial cells and platelets (50, 51), and impairs the fibrinolytic system by increasing plasminogen activator inhibitor-1 (PAI-1) (52). Inflammation is triggered by the activation of innate immune cells, releasing proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 β (IL-1 β), IL-6, IL-12, and IL-18 (53). Cytokines are the most important mediators of hemostasis activation during sepsis, which are mainly mediated by increased expression of TF (24). In addition, cytokines can further activate leukocytes, platelets, and endothelial cells, thereby promoting hemostasis activation (54). Notably, these cytokines not only activate procoagulant pathways and platelets but also downregulate the anticoagulant pathways of TFPI, protein C system, and AT, along with impairing fibrinolysis pathways through a sustained increase in PAI-1 (23, 24, 55, 56).

Collectively, these immune mechanisms result in a procoagulant state in the hemostatic balance during sepsis. More importantly, the activation of hemostasis also feeds back to the immune response (3). For example, coagulation proteases bind to protease-activated receptors expressed in innate immune cells, which can induce additional proinflammatory responses by releasing cytokines (23, 57). In this section, we focus on immune-related factors that induce hemostatic changes in sepsis; a more comprehensive discussion of these changes is not included in this review.

4 Pyroptosis-induced hemostasis activation in sepsis

In the early stages of sepsis, the host defense reaction induces pyroptosis, which participates in the innate immune response, and eliminates intracellular pathogens. However, excessive pyroptosis results in a dysregulated host immune response, systemic inflammatory reactions, and even organ failure (7). In addition, recent studies have revealed that the activation of pyroptosis and inflammasome are widely involved in hemostasis activation and immunothrombosis in sepsis.

4.1 Mechanisms of pyroptosis

The phenomenon of pyroptosis was discovered in 1992 (58), which reported that the death of macrophages infected with *Shigella flexneri* was caspase-1 dependent. In 2001, the concept of “pyroptosis” was first coined by Cookson and Brennan (59), who described the proinflammatory and lytic mode of programmed cell death. Pyroptosis is stimulated by PAMPs, such as LPS, flagellin, and type 3 secretion system (T3SS) structural proteins, and DAMPs, such as ATP, uric acid crystals, and high-mobility group box 1 (HMGB1) (60). Pyroptosis mechanisms include the canonical (caspase-1 dependent) and non-canonical pathways (caspase-4/5/11 dependent) (60–62).

In the canonical pyroptosis pathway, an inflammasome complex is formed. Five intracellular PRRs have been confirmed to form inflammasomes: the nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat-containing receptor (NLR) proteins, NLRP3 (NLR family pyrin domain containing 3), NLRP1, NLRC4 (NLR family caspase activation and recruitment domain [CARD] containing 4), as well as AIM2 (absent in melanoma 2) and pyrin (60, 63). Intracellular PRRs recognize pathogenic stimuli and bind to pro-caspase-1 through the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and assemble into inflammasome complexes that activate caspase-1 and then cleave pro-IL-1 β and pro-IL-18 (60). In these inflammasome complexes, NLRP1 and NLRC4 contain CARD domains, resulting in the ability to recruit pro-caspase 1 with or without ASC (64, 65). Significantly, the NLRP3 inflammasome is the most extensively studied inflammasome, and the assembly of the NLRP3 inflammasome requires two signals (60). The first is the priming signal, which entails the transcription of nuclear factor- κ B (NF- κ B)-mediated upregulation of pro-IL-1 along with NLRP3 (66). Stimulants for priming include ligands for TLRs, NLRs, and cytokine receptors (66). The second signal activates the NLRP3 inflammasome and is provided by ATP, potassium (K⁺) efflux, calcium signaling, cytosolic release of lysosomal cathepsins and reactive oxygen species (ROS), and certain bacterial toxins (60, 67).

The non-canonical pyroptosis is initiated after intracellular LPS directly binds and activates caspase-4/5 (in humans) or caspase-11 (in mice) (61, 62). Furthermore, intracellular LPS stimulation activates NLRP3 inflammasomes through the cleavage of the pannexin-1 channel and the release of ATP which causes P2X7 receptor activation and subsequent K⁺ efflux (68–70). Hence, the NLRP3 inflammasome is a pivotal connection between the canonical and non-canonical pathways of pyroptosis.

Subsequently, the activated caspases 1/4/5/11 cleave gasdermin D (GSDMD) into the C-terminal (22kDa) and N-terminal (31kDa) (71). The N-terminal of GSDMD then migrates and inserts itself into the cell membrane, oligomerizes, and forms pores (71). IL-1 β and IL-18 are cleaved into mature forms by caspase-1 and then released through the pores, which is additionally mediated by electrostatic filtering (71, 72). Pyroptotic cell death occurs through osmotic lysis, followed by the release of cell contents and a cascade of downstream responses (71, 73).

In addition to the canonical and non-canonical pathways of pyroptosis, new mechanisms triggering pyroptosis have been discovered. Caspase-3, a key marker of apoptosis activated by TNF-

α or chemotherapy drugs, can mediate pyroptosis by cleaving gasdermin E (GSDME) and forming pores on the cell membrane (74). Moreover, Caspase-8 cleaves GSDMD and GSDME, which are also involved in pyroptosis (75, 76).

4.2 Pyroptosis mediates the hemostasis activation in sepsis

Pyroptosis has displayed a prominent role in hemostasis imbalance and “immunothrombosis” in sepsis: it participates in regulating the release and activity of TF in macrophages and endothelial cells; GSDMD mediates NETs formation; pyroptosis in endothelial cells and platelets affects hemostasis; the release of proinflammatory cytokines promotes hemostasis activation.

4.2.1 Control of TF by pyroptosis

TF, the initiator of the extrinsic coagulation pathway, is a molecule prominently involved in the activation of pyroptosis-mediated coagulation in sepsis (10, 11, 77). Pyroptosis upregulates the release and activity of TF in sepsis, and the mechanisms are summarized in Figure 3.

Release of TF: Wu et al. showed that macrophage-derived microparticles containing TF are released after inflammasome activation and pyroptosis (11). They found that the activation of caspase-1 by T3SS rod proteins from *Escherichia coli* (*E. coli*) or activation of caspase-11 by intracellular LPS lead to GSDMD cleavage and pore formation, and subsequently triggered pyroptotic death and osmotic cell rupture. The TF then released in macrophages-derived microparticles results in the systemic activation of coagulation and subsequent death in mice (11). The caspase-1/11 activated mice showed increased coagulation time and thrombin-antithrombin complex level, and decreased fibrinogen and platelet counts, consistent with DIC in sepsis (11). Additionally, Rothmeier et al. reported that activated caspase-1 induced TF release. They found that in ATP-triggered macrophages, inflammasomes induced the activation of intracellular caspase-1 and calpain cysteine protease cascade, degrading filamin in macrophages and thereby severing bonds between the cytoskeleton and tissue factor (78). Furthermore, a recent study showed that in a bacterial infection mouse model (cecal ligation and puncture, CLP), activation of transmembrane protein 173 (TMEM173, also known as stimulator of interferon response cGAMP interactor, [STING]) triggered TF release and mediated coagulation activation, which relied on calcium release from the endoplasmic reticulum (77). Mechanistically, TMEM173 binding to inositol 1,4,5-trisphosphate receptor type 1 (ITPR1, also known as IP3R, the primary calcium release channel of the endoplasmic reticulum) in macrophages and monocytes stimulated with *E. coli* and *Streptococcus pneumoniae*, regulated the calcium influx, activated GSDMD by caspase-1/11 or caspase-8, culminating in TF release via pyroptosis (77). Collectively, these findings highlight that pyroptosis-involved caspase-1/8/11 and the GSDMD system play central roles in TF release.

Decryption of TF: Under physiological conditions, TF has low procoagulant activity, especially when expressed by blood cells and microparticles (79, 80). The decryption of TF is a post-translational process, increasing the procoagulant activity of TF by up to 100-fold

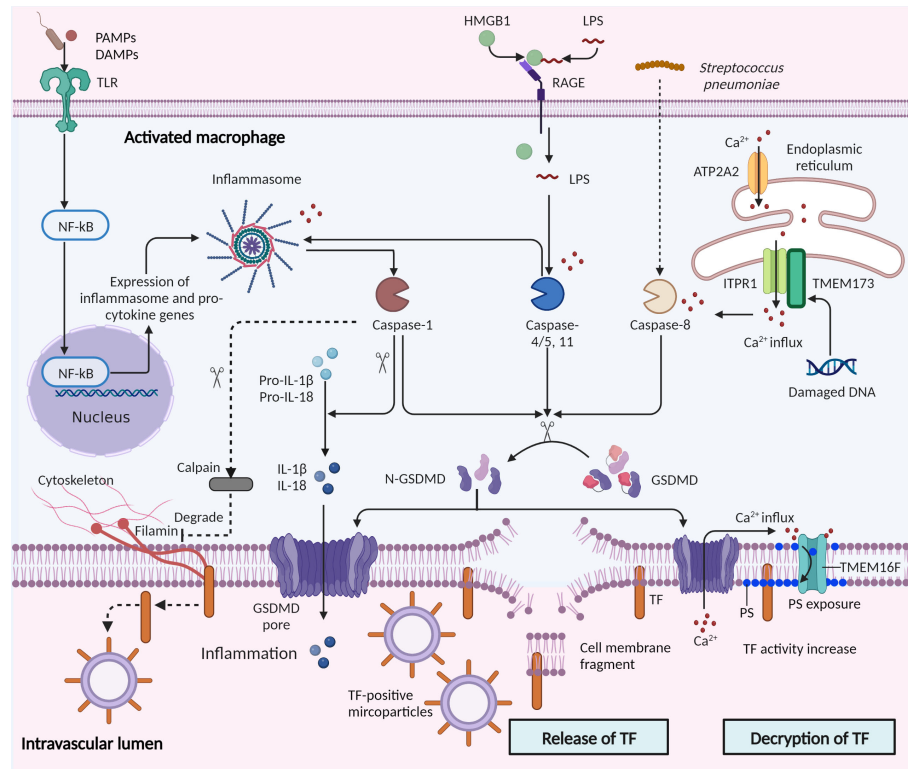


FIGURE 3

Control of tissue factor by pyroptosis in sepsis. Pyroptosis is stimulated by microbial infections and non-infectious stimuli, including host factors in sepsis. Canonical and non-canonical pyroptosis are both involved in tissue factor (TF) control. In canonical pyroptosis, pattern recognition receptors (PRRs) recognize pathogenic stimuli, and inflammasome complexes are assembled, which activate caspase-1 and then cleave pro-IL-1 β and pro-IL-18. Intracellular LPS directly binds and activates caspase-11. Subsequently, the activated caspases-1/11 cleave the gasdermin D (GSDMD) into the N-terminal of GSDMD (N-GSDMD), forming pores in the cell membrane. IL-1 β and IL-18 are then released through the pores and pyroptotic cell death occurs through osmotic lysis. TF is released in microparticles by the osmotic cell rupture in pyroptotic macrophages. In addition, caspase-1 may mediate TF release in another mechanism: activation of caspase-1 facilitates activation of calpain, degrading filamin in macrophages and thereby severing bonds between the cytoskeleton and TF. Furthermore, activation of transmembrane protein 173 (TMEM173) by infection-induced DNA damage mediates TF release. TMEM173 binds to inositol 1,4,5-trisphosphate receptor type 1 (ITPR1) in macrophages and monocytes, regulated the calcium influx, and activated GSDMD by caspase-1/11 or caspase-8. The GSDMD cleavage then triggers TF release through pyroptosis. Decryption of TF, a process increasing the procoagulant activity of TF, is triggered by GSDMD pores and subsequent phosphatidylserine (PS) exposure. GSDMD pores triggered calcium influx into the cytosol and promoted the exposure of PS to the outer cell membrane through TMEM16F, a calcium-dependent phospholipid scramblase. PAMPs, Pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; TLR, toll-like receptors; NF- κ B, nuclear factor- κ B; RAGE, receptor for advanced glycation end products; ATP2A2, ATPase sarcoplasmic/ER Ca²⁺ transporting 2.

(79, 81). This procoagulant phenotype characterizes TF-triggered “immunocoagulation”. One theory suggests that decryption is related to alterations of negatively charged phospholipids, such as phosphatidylserine, in the cell membrane (79). Recently, the decryption of TF has been shown to be related to inflammasome activation and pyroptosis. Yang et al. found that LPS activated caspase-11 and enhanced the decryption of TF by triggering the formation of GSDMD pores and subsequent phosphatidylserine (PS) exposure. Mechanistically, GSDMD pores triggered calcium influx into the cytosol and promoted the exposure of PS to the outer cell membrane through TMEM16F, a calcium-dependent phospholipid scramblase (10). Of note, the authors further utilized glycine and mannitol to prevent cell membrane rupture and cell lysis, and found that these did not affect TF’s procoagulant activity, suggesting that GSDMD-mediated TF decryption was independent of cell rupture in pyroptosis (10). In another study, high mobility group box 1 (HMGB1) from hepatocytes delivered extracellular LPS into the cytosol of macrophages and endothelial cells, where LPS induced caspase-11-dependent pyroptosis (82). Additionally, HMGB1

induced TF activation by promoting PS exposure through LPS-induced caspase-11 activation (83).

4.2.2 GSDMD in NETs formation

As we mentioned above, NETs contribute to immunothrombosis in sepsis in multiple ways. Previous studies demonstrated that NETs release involved two ways: NETosis, a novel neutrophil cell death pathway (84), and DNA extrusion mechanism from live cells (85). Recently, new research indicated that GSDMD pore also promoted NETs formation and release. Chen et al. found that pyroptosis induction in neutrophils and the non-canonical pyroptosis pathway activated NETs formation (86). Caspase-11 and GSDMD mediated NETs release which was dependent on neutrophil plasma membrane rupture and did not require factors usually associated with NETs release. Caspase-11 and GSDMD also participated in early features of NETosis, which was mediated by the nuclear membrane permeabilization and histone degradation driven by caspase-11 and GSDMD (86). Interestingly, a different mechanism of GSDMD-mediated NETs was found by Sollberger et al (87). The researchers

discovered that GSDMD was proteolytically activated by neutrophil proteases and, in turn, affected protease activation and nuclear expansion, which suggested that NETosis promoted by GSDMD was independent of inflammasome and caspase activation.

4.2.3 Pyroptosis in endothelial cell activation and dysfunction

Pyroptosis can occur in many cell types during sepsis, including endothelial cells, the major targets of inflammatory mediators, and innate immune cells. LPS induces non-canonical pyroptotic death of endothelial cells through caspase-11, resulting in endothelial injury associated with vascular leakage, increased leukocyte accumulation, and cytokine release in the lungs (88). Moreover, pyroptotic endothelial cells release microparticles in a caspase-11-dependent manner (88). Circulating histones, as DAMPs in sepsis, have been reported to induce pyroptosis through the NLRP3 inflammasome in human umbilical vein endothelial cells, which further results in the expression of endothelial adhesion molecules and an inflammatory response (89). Circulatory exosomes from COVID-19 patients trigger the NLRP3 inflammasome in cultured endothelial cells (90). Furthermore, Wang et al. found that monocytic microparticles activate endothelial cells *via* NLRP3 inflammasome, which induces phosphorylation of ERK1/2, activation of the NF- κ B pathway, and expression of the following cell adhesion molecules: intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin (91). These studies suggest that inflammasome activation and pyroptosis of endothelial cells induce endothelial activation and dysfunction, subsequently leading to inflammation and hemostasis activation.

4.2.4 Inflammasome activation in platelets

Although anucleate, platelets have a functional spliceosome and essential spliceosome factors, that are associated with constitutively present mRNA transcripts, including IL-1 β (92). Inflammasome activation in platelets shows “immunothrombosis” activities, which are mediated by IL-1 β production (93). Hottz et al. demonstrated that dengue virus infection leads to the activation of NLRP3 inflammasomes in platelets, triggering platelet shedding of IL-1 β -rich microparticles and increased expression of IL-1 β , which contributes to increased endothelial permeability (94). The platelet NLRP3 inflammasome is upregulated during platelet activation and thrombus formation *in vitro* (95); NLRP3 deficient platelets impaired hemostasis and thrombosis in mice (96). NLRP3 affected platelet α IIB β 3 outside-in signal transduction, an essential process for hemostasis and thrombosis, which might be mediated by IL-1 β , as significantly reduced IL-1 β release was found in NLRP3-deficient platelets (96). Furthermore, in thrombin activated-platelets, the caspase-3/GSDME pathway was upregulated, further regulating platelet integrin α IIB β 3 activation (97).

4.2.5 Pyroptosis-released cytokines in hemostasis activation

During pyroptosis, IL-1 β and IL-18, both important inflammatory cytokines in sepsis, are released (98). These cytokines, together with the downstream inflammatory mediators, such as IL-6 and TNF- α , further result in coagulation upregulation, leukocyte activation, endothelial activation and dysfunction, and platelets

activation and aggregation (23, 54, 99–101). All these responses cumulatively lead to hemostasis activation and thrombus formation, accelerating the development of DIC in sepsis.

4.3 Potential therapeutic strategies targeting pyroptosis

Although excessive hemostasis activation in sepsis is associated with organ dysfunction and death, there have been long-standing debates over the efficacy of anticoagulant therapy in managing sepsis (102, 103), regarding the timing and the hemorrhagic complications. Theoretically, the most logical timing for anticoagulant treatment is the prothrombotic stage of the hemostasis change in sepsis. Inappropriate hemostasis intervention during “immunocoagulation” in host defense against pathogens, or during fibrinolytic DIC, will lead to the aggravation of sepsis, which should also be taken into consideration in the intervention of pyroptosis-induced hemostasis imbalance. Based on the mechanism of pyroptosis-induced hemostasis activation described earlier, potential inhibitors that may prevent extensive hemostasis activation in sepsis are listed below

4.3.1 Inhibition of inflammasome activation

Previous studies have shown that inhibition of NLRP3 inflammasome activation results in protective effects against sepsis (104). In particular, MCC950, which inhibits NLRP3 by directly targeting the NLRP3 ATP-hydrolysis motif (105), attenuates multi-organ injuries in CLP rats (106), highlighting the potential of NLRP3 inhibitors in treating sepsis. Oridonin (107), tranilast (108), CY-09 (109), itaconate (110), etc. are further compounds that target NLRP3.

4.3.2 Caspase-related inhibitors

Broad-spectrum caspase inhibitors Z-VAD-FMK and VX-166 can reduce IL-1 β and IL-18 release and show significant therapeutic effects against sepsis in human patients and rodent models (111, 112). Ac-FLTD-CMK inhibits GSDMD cleavage by caspases-1/4/5/11, thereby suppressing canonical and non-canonical pyroptosis (113). Z-IETD-FMK blocks caspase-8-mediated GSDMD cleavage and prevents TF release in bone-marrow-derived macrophages (BMDMs) (77). oxPAPC, a caspase-4/11-targeting inhibitor, competes with LPS binding and consequently inhibits LPS-induced pyroptosis and septic shock (114). Ac-YVAD-CMK blocks caspase-1 activity, inhibiting IL-1 β release and the generation of thrombo-inflammatory microparticles in BMDMs (78).

4.3.3 Inhibition of GSDMD

Disulfiram, a drug used to treat alcohol addiction, as an inhibitor of pore formation by GSDMD, blocks pyroptosis and cytokine release in cells and prevents LPS-induced death in mice (115). LDC7559, a small molecule based on the pyrazolo-oxazepine scaffold, specifically binds GSDMD and blocks the activity of the GSDMD N-terminal (87).

In addition, blockers of other molecules or events regulating upstream and downstream inflammasome activation or pyroptosis also exhibit potential roles for the control of coagulation in sepsis (116–118). These include preventing pyroptosis-induced membrane rupture, inhibiting calcium influx, and preventing cytosolic delivery

of LPS with heparin or by antagonizing HMGB1 (116–118). Although may be potential treatments for sepsis, unfortunately, they have not been translated into clinical sepsis, and more research is needed to convert promising experimental results into effective clinical drugs.

5 Conclusion

Sepsis is characterized by excessive hemostasis activation, multiple organ dysfunction, and high mortality, and remains a major challenge for basic and clinical research. Understanding the mechanisms of pyroptosis-induced hemostatic imbalance in sepsis increases our understanding of the characteristics and consequences of the host defense response to pathogens and brings new insights into sepsis-induced coagulopathy. The regulation of TF release and decryption in pyroptosis is a key discovery in “immunocoagulation,” as it links the key effectors of inflammation and coagulation. In addition, pyroptosis and inflammasome activation pathways contribute to NETs formation and activation of endothelial cells and platelet, which are related to hemostasis activation in sepsis. Elucidation of pyroptosis-induced hemostatic activation pathways and their implications in thrombosis might reveal new therapeutic approaches for septic DIC and lethal sepsis.

However, concerns and questions remain unaddressed. The pyroptosis pathways in hemostatic change are interconnected with other events in the development of sepsis, which means that intervention during hemostasis will affect the host response and could aggravate the disease. Hence, the focus should be on further in-depth comprehension of intermediate connections to achieve precise coagulation therapy. Moreover, proper therapies tailored to the prothrombotic stage of DIC should be established in the future.

Author contributions

CZ and YLu reviewed the literature, wrote the draft of the manuscript, and prepared figures. YLi helped in the evaluation of

the literature. XM designed and supervised the work. All authors contributed to the review and approved the submitted version. The research was supported by the National Nature Science Foundation of China (Grant No.82172130) and Program of Education Department of Liaoning Province (LJKMZ20221144).

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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