



Monocyte Tissue Factor Expression: Lipopolysaccharide Induction and Roles in Pathological Activation of Coagulation

Ana T. A. Sachetto¹ Nigel Mackman¹

¹Division of Hematology, Department of Medicine, UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Address for correspondence Nigel Mackman, PhD, Department of Medicine, 116 Manning Drive, University of North Carolina at Chapel Hill, 8004B Mary Ellen Jones Building, Chapel Hill, NC 27599, United States (e-mail: nmackman@med.unc.edu).

Thromb Haemost 2023;123:1017–1033.

Abstract

The coagulation system is a part of the mammalian host defense system. Pathogens and pathogen components, such as bacterial lipopolysaccharide (LPS), induce tissue factor (TF) expression in circulating monocytes that then activates the coagulation protease cascade. Formation of a clot limits dissemination of pathogens, enhances the recruitment of immune cells, and facilitates killing of pathogens. However, excessive activation of coagulation can lead to thrombosis. Here, we review studies on the mechanism of LPS induction of TF expression in monocytes and its contribution to thrombosis and disseminated intravascular coagulation. Binding of LPS to Toll-like receptor 4 on monocytes induces a transient expression of TF that involves activation of intracellular signaling pathways and binding of various transcription factors, such as c-rel/p65 and c-Fos/c-Jun, to the TF promoter. Inhibition of TF in endotoxemia and sepsis models reduces activation of coagulation and improves survival. Studies with endotoxemic mice showed that hematopoietic cells and myeloid cells play major roles in the activation of coagulation. Monocyte TF expression is also increased after surgery. Activated monocytes release TF-positive extracellular vesicles (EVs) and levels of circulating TF-positive EVs are increased in endotoxemic mice and in patients with sepsis. More recently, it was shown that inflammasomes contribute to the induction of TF expression and activation of coagulation in endotoxemic mice. Taken together, these studies indicate that monocyte TF plays a major role in activation of coagulation. Selective inhibition of monocyte TF expression may reduce pathologic activation of coagulation in sepsis and other diseases without affecting hemostasis.

Keywords

- ▶ coagulation
- ▶ inflammation
- ▶ lipopolysaccharide
- ▶ monocytes
- ▶ tissue factor

Bacterial Infection and Coagulation

Bacterial infections represent a major challenge to invertebrate and vertebrate animals. Detection of pathogen-associated molecular patterns (PAMPs), such as components of

bacteria, as well as damage-associated molecular patterns (DAMPs), such as molecules released by damaged or dying cells, lead to activation of the host immune system.^{1,2} The coagulation system is part of the host immune system and is activated during bacterial infection. Formation of a clot limits

received
September 3, 2021
accepted after revision
May 8, 2023
accepted manuscript online
May 11, 2023
article published online
June 19, 2023

DOI <https://doi.org/10.1055/a-2091-7006>.
ISSN 0340-6245.

© 2023. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)
Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

dissemination of pathogens.³ Bacterial lipopolysaccharide (LPS) (also known as endotoxin) is a major component of the outer membrane of gram-negative bacteria. This PAMP alerts the host to invading gram-negative bacteria and is a potent activator of the host immune and coagulation systems.⁴

LPS Activation of Coagulation in Invertebrates and Vertebrates

Invertebrate animals, such as horseshoe crabs, are highly sensitive to LPS.^{3,5} The horseshoe crab has existed for 200 million years. In 1885, William H. Howell reported that hemolymph of the Atlantic horseshoe crab *Limulus polyphemus* could form a gel-like clot.^{6,7} Jack Levin and Frederik Bang showed that LPS activated *L. polyphemus* coagulation and this involved procoagulant proteins present in hemocytes (arthropod blood cells analogous to mammalian monocytes).^{8,9} Two decades later, Sadaaki Iwanaga characterized the components of the limulus clotting system and showed that clot formation occurs in a waterfall cascade-like pattern that is similar to the coagulation protease cascade in mammals¹⁰ (►Fig. 1A). Limulus hemocytes were found to contain three serine protease zymogens and one clottable protein. Exposure of hemocytes to LPS induces the release of these proteins into the hemolymph. LPS then activates factor C which subsequently activates factor B. Next, factor B acti-

vates the pro-clotting enzyme to clotting enzyme that converts coagulogen into coagulin, forming an insoluble gel.

The limulus clotting system has been developed into the limulus amoebocyte lysate assay. This is a highly sensitive assay that is used to detect and quantify LPS in reagents and biological samples. It consists of an extract of hemocytes from *L. polyphemus*.

Mammals maintain hemostasis using a coagulation protease cascade that results in the formation of crosslinked fibrin (►Fig. 1B). This cascade was first described in 1964.^{11,12} Tissue factor (TF) is constitutively expressed by perivascular cells around blood vessels and by epithelial cells at body surfaces and forms a hemostatic envelope to limit bleeding.¹³ Briefly, TF is a transmembrane receptor and cofactor for factor (F) VIIa.¹⁴ The TF-FVIIa complex activates the coagulation cascade by cleavage of both FIX and FX. Next, the prothrombinase complex (FXa-FVa) activates prothrombin to thrombin. Thrombin is the central protease in the coagulation cascade and cleaves fibrinogen to fibrin monomers.

LPS induces TF expression in monocytes, and this then activates blood coagulation (►Fig. 1B). It takes some time to induce TF expression in monocytes and therefore this TF does not play a role in hemostasis but rather contributes to the host defense by forming clots at sites of infection that help prevent the spread of pathogens.

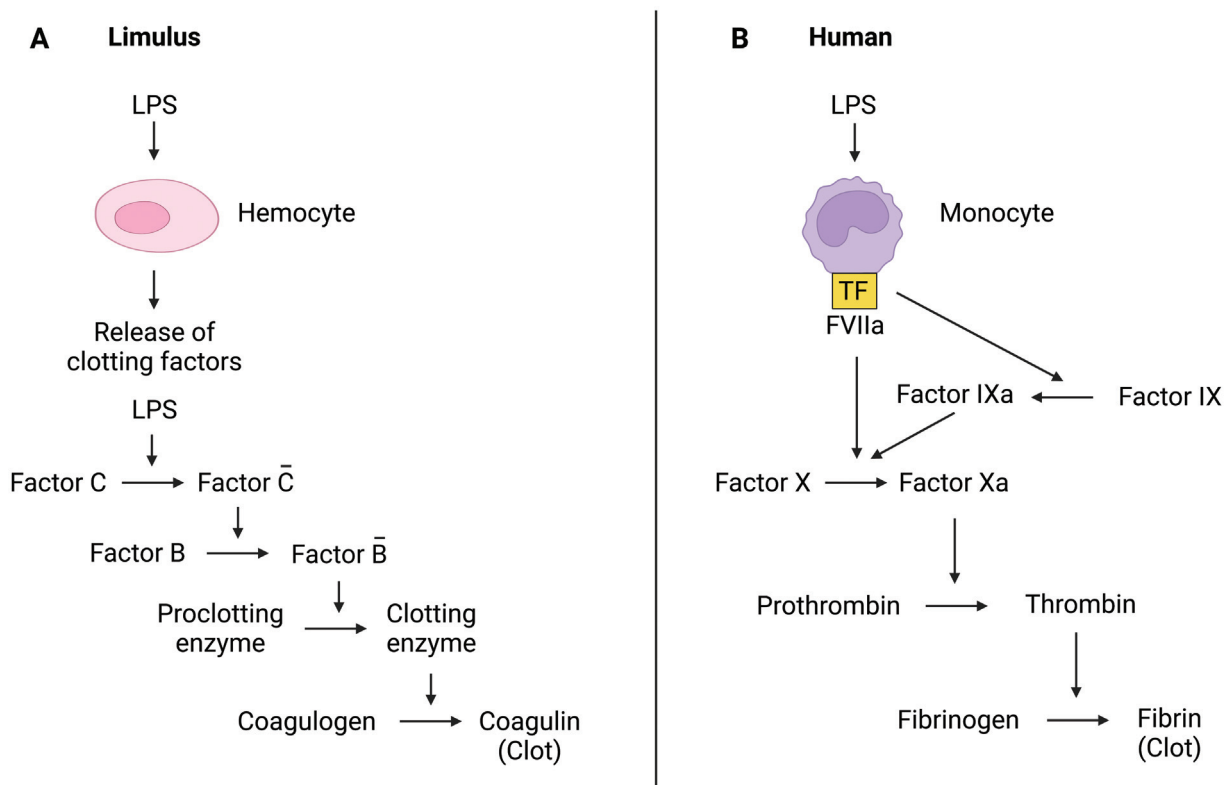


Fig. 1 Bacterial lipopolysaccharide induces clotting reactions in different organisms. (A) Hemocytes from *Limulus* horseshoe crabs exposed to lipopolysaccharide (LPS) release serine proteases (factor C, factor B, and pro-clotting enzyme) and a clottable protein (coagulogen) into the hemolymph. LPS activates factor C that activates factor B and converts the pro-clotting enzyme to a clotting enzyme leading to the conversion of coagulogen into coagulin and the formation of a clot (insoluble gel). (B) LPS induces tissue factor (TF) in human monocytes and this activates blood coagulation. The TF-factor (F) VIIa complex cleaves FIX and FX. The prothrombinase complex (FXa-FVa) activates prothrombin to thrombin that converts fibrinogen into fibrin, forming a clot.

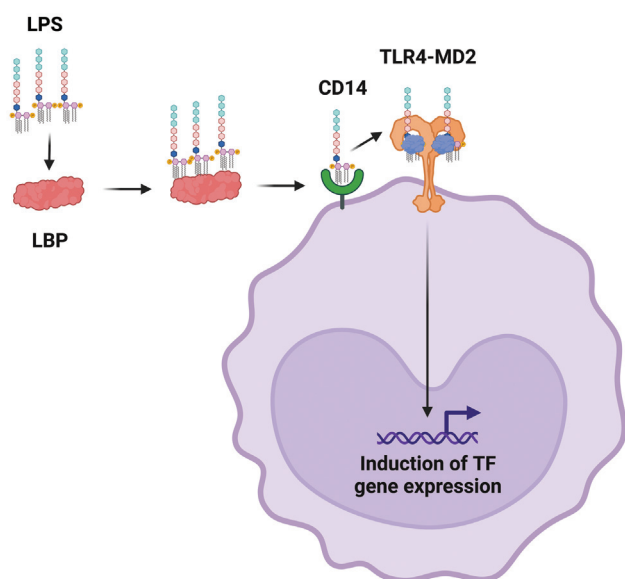


Fig. 2 Mechanism of induction of tissue factor (TF) by bacterial lipopolysaccharide (LPS) in human monocytes. LPS-binding protein (LBP) binds to LPS and transfers LPS to the monocyte receptor CD14. LPS is transferred to the Toll-like receptor 4 (TLR4)–MD-2 complex and this activates intracellular signaling pathways and transcription factors that lead to TF gene expression.

LPS Activation of Monocytes

The presence of LPS in the bloodstream is called endotoxemia. LPS-binding protein (LBP) present in blood binds to LPS and transfers it to CD14, which is a receptor expressed by monocytes^{15–19} (►Fig. 2). Next, LPS binds to Toll-like receptor 4 (TLR4), which was shown to be the LPS signaling receptor on monocytes and macrophages.²⁰ TLR4 forms heterodimers with MD-2²¹ (►Fig. 2). LPS binding to the TLR4–MD-2 complex activates a variety of intracellular signaling pathways that result in activation of different transcription factors, such as activator protein 1 (AP-1), and nuclear translocation of nuclear factor- κ B (NF- κ B). This leads to the expression of TF and proinflammatory cytokines (►Fig. 2).

Endotoxemia and Sepsis

Injection of a low dose of LPS into human volunteers is used to study different components of the host immune system, such as inflammation and coagulation. In addition, injection of LPS into animals leads to endotoxemia that mimics some aspects of gram-negative bacterial infection.

Uncontrolled infections may lead to the presence of pathogens in the bloodstream and sepsis, which is a body's extreme response to an infection. Sepsis is a public health issue affecting 30 million people per year worldwide.²² It is associated with activation of the coagulation system, microvascular thrombosis, and disseminated intravascular coagulation (DIC).²³ Consumption of coagulation factors during sepsis can lead to bleeding, which can present as petechiae

(small red spots in the skin). There are different animal models of sepsis, including a baboon model that uses a LD100 dose of *Escherichia coli*.

Timeline of Studies of LPS Induction of TF Expression in Monocytes

►Fig. 3 shows a timeline of the major methodological tools and scientific discoveries in the field of LPS induction of TF expression in monocytes.

LPS Induction of TF Activity in Monocytes

In 1971, Lerner et al showed that LPS induced procoagulant activity in human leukocytes isolated from healthy individuals.²⁴ This activity was dependent on the presence of FVII, which suggested it was TF. Four years later, Rivers et al²⁵ demonstrated that monocytes within the leukocyte population were responsible for the LPS-stimulated procoagulant activity and confirmed that it was TF. In 1983, Semeraro et al showed that monocytes alone exhibited increased TF activity when stimulated with LPS.²⁶ In 1994, we and another group showed that LPS induction of TF activity in human monocytes was mediated by the LBP–CD14 pathway.^{27,28}

Other studies have shown that different cell populations and stimuli increase LPS-induced TF expression in human monocytes. Two studies by Osterud and colleagues showed that LPS induction of TF in monocytes was enhanced by platelet activating factor, granulocytes, and platelets.^{29,30} In addition, they reported that platelet lysate supernatants and purified platelet factor 4 enhanced TF activity in LPS-stimulated human monocytes.³¹ Another group also observed that platelet activating factor enhanced TF activity after LPS stimulation of human monocytes.³² Landsem et al³³ analyzed the role of complement and CD14 in LPS and *E. coli* induction of TF in a human whole blood model. As expected, inhibition of CD14 reduced LPS induction of TF mRNA and protein expression in monocytes. Surprisingly, a similar reduction of LPS induction of TF expression was observed using the C3 blocking peptide compstatin, which indicated that activation of the complement cascade enhanced LPS induction of TF expression in monocytes. More recently, it was shown that an anti-C5 antibody did not affect LPS induction of TF protein expression on human monocytes but reduced TF activity by inhibiting phosphatidylserine (PS) exposure.³⁴

It is well known that many commercial-grade reagents, including recombinant proteins, are contaminated with LPS.³⁵ Indeed, induction of TF in monocytes by recombinant cytokines may be due to LPS contamination. For instance, it was reported that interleukin (IL)-6 and IL-8 induce TF expression in human monocytes but the LPS inhibitor polymyxin B was not used to exclude the possibility that this was due to LPS contamination.³⁶ We found that recombinant tumor necrosis factor α (TNF- α) induced TF expression in human monocytes but this activity was abolished with an anti-CD14 antibody suggesting that it was due to LPS contamination of the TNF- α .²⁸

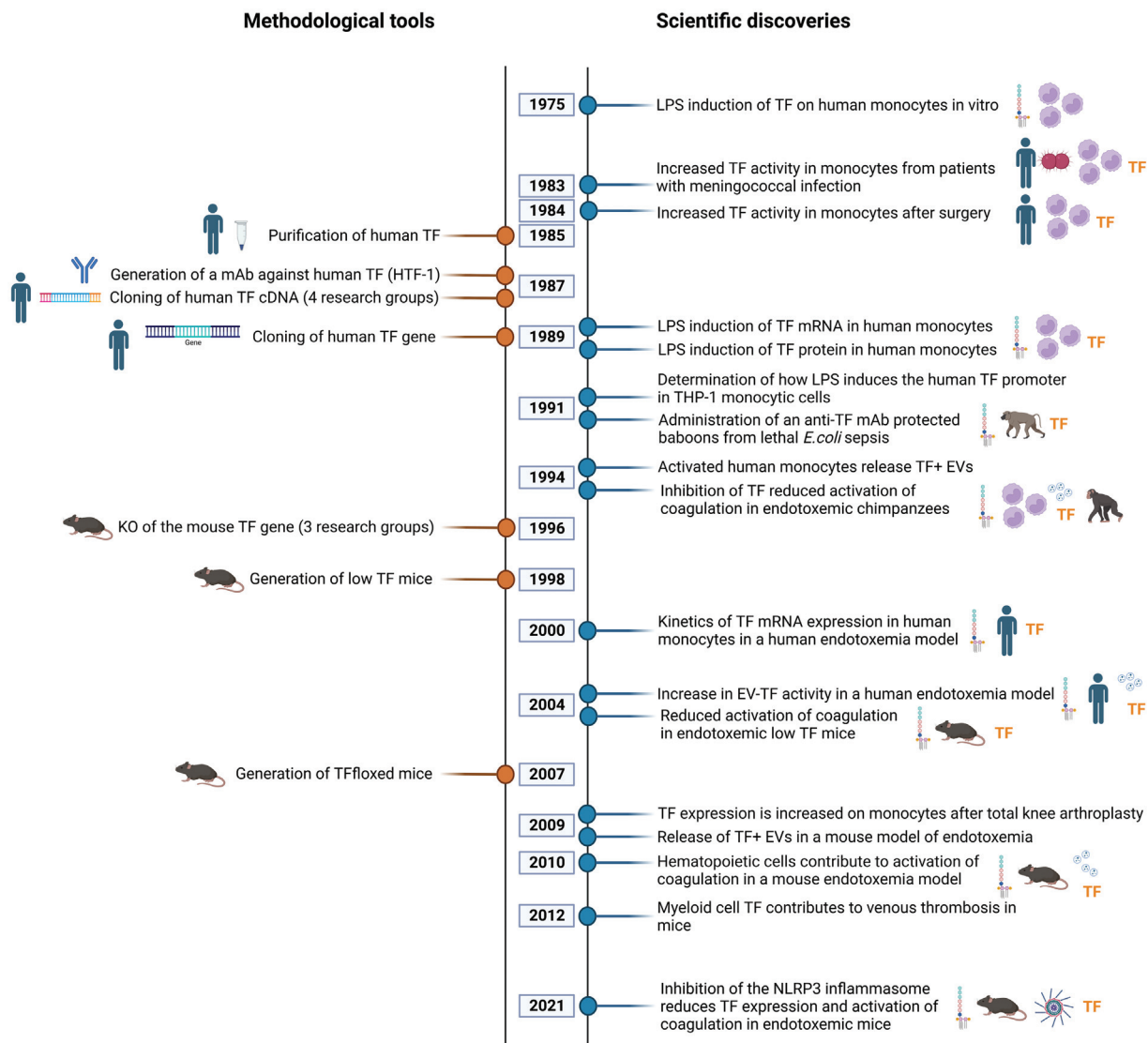


Fig. 3 Timeline of the major methodological tools and scientific discoveries in the field of lipopolysaccharide induction of tissue factor. DIC, disseminated intravascular coagulopathy; *E. coli*, *Escherichia coli*; EVs, extracellular vesicles; KO, knockout; LPS, lipopolysaccharide; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; TF, tissue factor.

LPS Induction of TF Protein in Monocytes

In 1985, Broze and colleagues³⁷ purified human TF (→ Fig. 3). This work allowed the generation of anti-TF antibodies and also facilitated the cloning of the human TF complementary DNA (cDNA). In 1987, Carson and colleagues³⁸ reported the generation of the first mouse anti-human TF monoclonal antibody called HTF-1. This antibody is commercially available and is the gold standard antibody used by many investigators for TF studies.^{39,40} Subsequently, several groups generated a variety of monoclonal antibodies against human TF.^{41–43} The human TF protein is composed of an extracellular domain, a transmembrane domain, and a cytoplasmic domain.⁴⁴ Mapping studies showed that these anti-human TF monoclonal antibodies bound to three different regions of the TF protein^{43,45–47} (→ Fig. 4). First, there was a group of antibodies that bound to the N-terminal region of the extracellular domain of TF and competed with binding of

FVII/FVIIa. A second group of antibodies bound to the C-terminal region of the extracellular domain of TF and bound to either free TF or TF complexed with FVII/FVIIa. These antibodies interfered with substrate binding. Finally, the last group of antibodies bound to the C-terminal region of the extracellular domain of TF close to the transmembrane membrane and did not inhibit TF procoagulant activity but inhibited TF signaling.

In 1989, Drake et al used a cocktail of three anti-TF antibodies to stain TF in different tissues and observed a strong signal on LPS-stimulated human monocytes but not neutrophils.¹³ Other studies have shown TF protein expression on LPS-stimulated monocytes by flow cytometry.^{39,48} There has been some controversy about the specificity of anti-human TF monoclonal antibodies. Basavaraj et al³⁹ evaluated the ability of five anti-human TF monoclonal antibodies, including HTF-1, to bind to unstimulated and LPS-stimulated human monocytes. Three of the antibodies

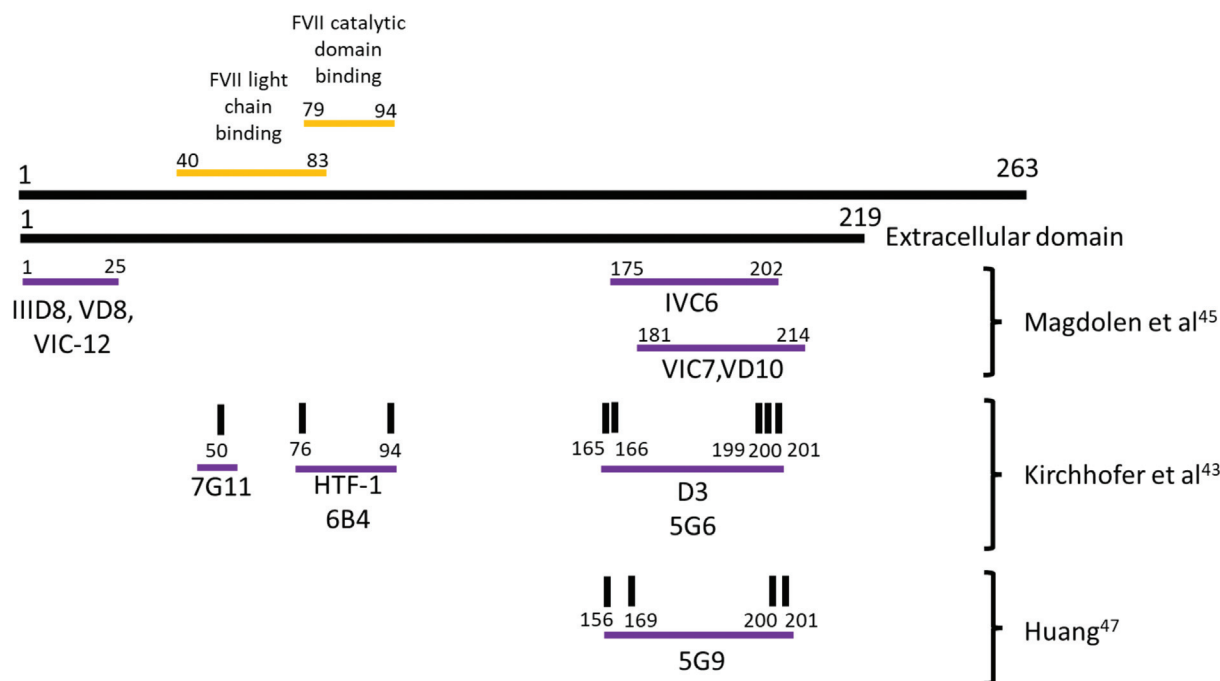


Fig. 4 Binding sites of different anti-human tissue factor (TF) mouse monoclonal antibodies. Full length TF and the extracellular domain are shown as black lines. The FVII light chain and FVII catalytic domain-binding sites are shown as orange lines. Magdolen et al⁴⁵ used a combination of truncated TF proteins and synthetic peptides to map the epitopes of different antibodies. Kirchhofer et al⁴³ and Huang et al⁴⁷ used a combination of mutagenesis and structural data to map the epitopes of different antibodies. Horizontal purple lines indicate the different binding regions and vertical black lines indicate the individual amino acid contact points of the different antibodies.

(HTF-1, TF9-10H10, and VD8) bound to LPS-stimulated monocytes but not to unstimulated monocytes, whereas VIC7 and CLB/TF-5 bound to unstimulated monocytes suggesting non-specific binding since these cells do not express TF.

LPS Induction of TF Gene and mRNA Expression in Monocytes and Monocytic Cells

In 1987, four groups cloned the human TF cDNA^{44,49-51} (►Fig. 3). This opened the door to studies on TF mRNA expression in different cell types, including monocytes. Gregory et al⁵² were the first to analyze LPS stimulation of TF gene, mRNA, and activity in human monocytes. They used a nuclear runoff assay to measure TF gene expression and found that LPS induced a transient expression of the TF gene with a maximal level at 1 hour. Next, they used northern blotting to show that LPS induced a transient induction of TF mRNA with a maximal level at 4 hours. Finally, they measured TF activity and observed a transient increase with a maximal level at 6 hours. Other studies have shown LPS transiently induces TF expression in monocytes.^{48,53} We analyzed LPS induction of TF gene expression, mRNA expression, and activity in THP-1 cells,⁵¹ which is a human monocytic cell line derived from a patient with acute monocytic leukemia. Like data with human monocytes, we observed that LPS stimulated a transient expression of TF with maximal levels of TF gene expression, mRNA expression, and activity at 1, 2, and 5 hours, respectively. These studies

indicate that TF expression in monocytes and monocytic cells is tightly regulated.

Interestingly, TF mRNA contains four AUUUA elements in the 3'-untranslated region. These elements were previously shown to regulate mRNA stability of TNF- α mRNA.⁵⁴ Gregory et al⁵² measured the stability of TF mRNA in LPS-stimulated human monocytes and found that it had a half-life of approximately 1.5 hours. We also found that TF mRNA was unstable in THP-1 cells.⁵¹ These results indicate that TF mRNA is rapidly degraded to limit the amount of TF protein that is expressed on LPS-stimulated human monocytes and monocytic cells.

LPS Induction of the Human TF Promoter

In 1989, we cloned the human TF gene.⁵¹ This permitted studies of the TF promoter. In 1991, we cloned various lengths of the promoter into a plasmid upstream of the firefly luciferase reporter gene and transfected these constructs into THP-1 cells to identify DNA sequence elements responsible for basal and LPS induction of the TF promoter.⁵⁵ The largest fragment of the promoter was -2,106 to +121 bp and the smallest fragment was -111 to +121 bp. We found that five Sp1 sites were required for basal TF promoter expression in THP-1 cells⁵⁶ (►Fig. 5). Next, we analyzed LPS induction of the TF promoter. We found that a 56 bp region in the promoter containing two AP-1 binding sites and one NF- κ B site was required for LPS induction of the TF promoter in THP-1 cells (►Fig. 5). We called this 56 bp region

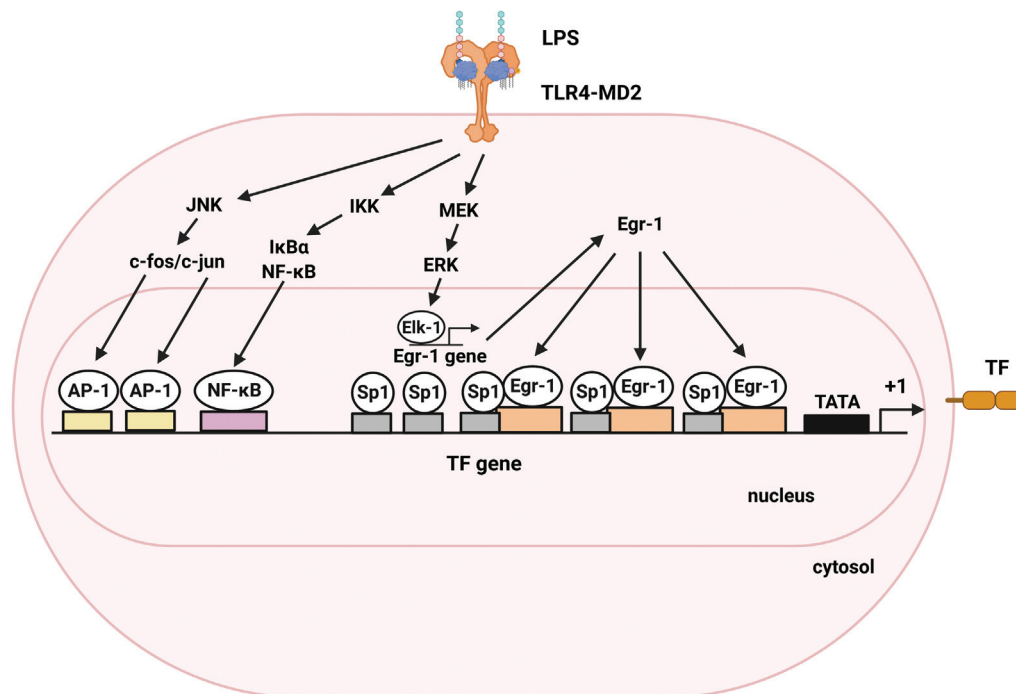


Fig. 5 DNA sequence elements responsible for basal and lipopolysaccharide (LPS) induction of tissue factor (TF) promoter. Basal expression of the TF promoter is mediated by 5 Sp1 sites. A 56 bp region in the promoter called LPS response element (LRE) mediates LPS induction of the TF promoter and contains two activator protein 1 (AP-1) binding sites and one nuclear factor- κ B (NF- κ B) site. Two Ets binding sites are also required for LPS induction and are located in the LRE. The transcription factor Egr-1 is induced by LPS and contributes to LPS activation of the TF promoter.

the LPS response element. Interestingly, the NF- κ B site (5'-CGGATTTCC-3') in the TF promoter did not fit the consensus for NF- κ B sites (5'GGGRNYYCC-3') and rather than binding to the prototypic p50/p65 heterodimer we found that it bound to a c-Rel/p65 heterodimer.⁵⁷ Another group showed that LPS induction of the TF promoter in THP-1 cells was mediated by binding of c-Rel/p65 to the NF- κ B site and AP-1 proteins to the AP-1 sites.⁵⁸ The AP-1 sites have been reported to bind various members of the c-Jun and c-Fos families.^{56,58} In addition, we found that the transcription factor Egr-1 was induced by LPS stimulation of THP-1 cells and contributed to LPS activation of the TF promoter (**Fig. 5**).^{56,59} LPS activation of the MEK-ERK signaling pathway activated the transcription factor Elk1 that induced expression of Egr-1, which then bound to the TF promoter. Finally, DNase I footprinting of THP-1 cells was used to identify sites of protein-DNA interaction in the TF promoter between -383 and the cap site. This study showed that two LPS-inducible elements were located at -231 to -172 and -85 to -52. The upstream site contained binding sites for c-Jun and Ets-1 and Ets-2 (-192 to -177) and the downstream site bound Egr-1 (-85 to -52) (**Fig. 5**).⁶⁰ One study investigated the effect of pentraxin-3 on LPS induction of TF expression in human monocytes.⁶¹ Pentraxin-3 is increased in septic shock. Pentraxin-3 increased levels of c-Rel/p65 in the nucleus and TF expression in monocytes. These studies indicate that numerous intracellular signaling pathways and transcription factors mediate basal and LPS induction of the TF promoter in human monocytic cells.

Inhibitors of LPS Induction of TF Expression in Monocytes

Many studies have used inhibitors to identify intracellular signaling pathways and transcription factors that are required for LPS induction of TF expression in monocytes (**Table 1**). Ternisien et al demonstrated that three protein kinase C inhibitors (H7, staurosporine, and calphostin)⁶² and two protein tyrosine kinase inhibitors (herbimycin A and genistein)⁶³ decreased LPS-induced TF mRNA, protein, and activity in human monocytes. These inhibitors did not affect TF mRNA stability indicating that they prevented LPS induction of TF gene expression.

LPS stimulation of leukocytes induces the production of reactive oxygen species (ROS) that act as DAMPs. Several studies analyzed the effect of different antioxidants on LPS induction of TF expression in monocytes. In 1997, Polack et al⁶⁴ demonstrated that two scavengers of ROS (*N*-acetyl cysteine and pyrrolidine dithiocarbamate) decreased LPS induction of TF expression in human monocytes. In addition, two NADPH oxidase inhibitors (phenylarsine oxide and diphenyleneiodonium) and two nitric oxide synthetase inhibitors (diphenyleneiodonium and *L*-*N*-methyl arginine) decreased LPS induction of TF expression. Interestingly, *N,N'*-dimethyl- γ - γ -dipyridylum dichloride, which generates superoxide, and sodium nitrosylpentacyanoferrate, which generates nitric oxide, both restored LPS induction of TF expression in the presence of the inhibitors suggesting that these two radicals participate in the induction of TF expression.

Table 1 Inhibitors of lipopolysaccharide induction of tissue factor expression in monocytes

Agent	Target	Reference
H7	Protein kinase C	62
Staurosporine	Protein kinase C	62
Calphostin	Protein kinase C	62
Herbimycin A	Protein tyrosine kinase	63
Genistein	Protein tyrosine kinase	63
N-acetyl cysteine	Reactive oxygen species	64
Pyrrolidine dithiocarbamate	Reactive oxygen species	64
Phenylarsine oxide	NADPH oxidase	64
Diphenyleneiodonium	Nitric oxide synthetase	64
L-N-methyl arginine	Nitric oxide synthetase	64
Succinobucol (AGI-1067)	Reactive oxygen species	65
AGI-1095	Reactive oxygen species	65
Resveratrol	Reactive oxygen species	66
N ^α -tosylphenylalanyl chloromethyl ketone	NF-κB	67
N ^α -tosyl-L-lysine chloromethyl ketone	NF-κB	67
Cyclosporine A	NF-κB	68
Acetylsalicylic acid	NF-κB	69,70
Sodium salicylate	NF-κB	69,70
Dipyridamole	Protein kinase A	72
Pentoxifylline	Protein kinase A	71,74
Iloprost	Protein kinase A	73
Forskolin	Protein kinase A	53,75
Iso-butyl-methyl-xanthin	Protein kinase A	53
Dibutyryl-cAMP	Protein kinase A	53,75
Pravastatin	RhoA	79

Probuco is an antioxidant drug that was used experimentally to treat patients with cardiovascular disease but had side effects. A new class of probuco derivatives was made that include succinobucol (also called AGI-1067) and AGI-1095. We found that these drugs inhibited LPS induction of TF mRNA and activity in THP-1 cells.⁶⁵ Interestingly, AGI-1067 and AGI-1095 inhibited the redox-sensitive kinase, apoptosis signal-regulating kinase-1 (ASK1), and the mitogen-activated protein kinases (MAPKs) p38, ERK1/2, and JNK1/2. This led to a reduction in phosphorylated AP-1 and synthesis of EGR-1 and reduced TF gene transcription without affecting nuclear translocation of NF-κB.⁶⁵ The phytochemical resveratrol found in red wine has antioxidant activity. Pendurthi et al⁶⁶ showed that resveratrol inhibited LPS induction of TF expression in human monocytes without affecting binding of c-Fos/c-Jun or c-Rel/p65 to the TF promoter. These findings demonstrate that antioxidants can affect different intracellular pathways and interfere with LPS induction of TF expression in monocytes.

Activation of NF-κB requires degradation of IκBα that is mediated by the proteasome. In 1994, we showed that two

proteasome inhibitors (N^α-tosylphenylalanyl chloromethyl ketone and N^α-tosyl-L-lysine chloromethyl ketone) blocked LPS induction of TF expression in THP-1 cells.⁶⁷ These inhibitors prevented nuclear translocation of c-Rel/p65. Interestingly, the immunosuppressant drug cyclosporine A also inhibited LPS induction of TF expression in human monocyte/macrophages by preventing nuclear translocation of c-Rel/p65.⁶⁸ We and another group showed that sodium salicylate and acetylsalicylic acid inhibited LPS induction of TF expression in human monocytes and THP-1 cells by preventing nuclear translocation of c-Rel/p65.^{69,70} However, two other nonsteroidal anti-inflammatory drugs (ibuprofen and indomethacin) did not affect LPS induction of TF expression.

Drugs and agents that increase intracellular cyclic AMP and activation of protein kinase A have been shown to inhibit LPS induction of TF expression in monocytes and THP-1 cells. These include the antiplatelet drug dipyridamole, the methylxanthine derivative pentoxifylline, the stable prostacyclin analog iloprost, prostaglandin E1, forskolin, iso-butyl-methyl-xanthin, and dibutyryl-cAMP.^{53,71–74} We showed that forskolin and dibutyryl-cAMP did not affect LPS-induced

nuclear translocation of c-Rel/p65 but blocked the functional activity of the complex in the nucleus.⁷⁵

Various statins have been reported to inhibit LPS induction of TF expression in monocytes and the monocytic cell line U937.^{76–79} It was shown that statins have different potencies against NF- κ B and this paralleled their ability to inhibit TF expression. In addition, Nagata et al⁷⁹ showed that ceristatin and pravastatin inhibited the geranylgeranylation of RhoA. Furthermore, the ROCK inhibitor Y-27632 reduced LPS induction of TF expression in monocytes suggesting that statins reduce TF expression by inhibiting the activation of RhoA kinase.

Finally, we analyzed the role of phosphatidyl-inositol 3-kinase (PI3K) in LPS induction of TF expression in THP-1 cells.⁸⁰ PI3K is an enzyme that modifies membrane inositol lipids and this allows binding of various signaling proteins, such as Akt. LPS activates the PI3K–Akt pathway. We used two different inhibitors of PI3K (wortmannin and Ly294002) and found that they enhanced LPS induction of TF expression.⁸⁰ We showed that the PI3K–Akt pathway negatively regulated the Raf1–MEK–ERK1/2 signaling pathway, which is required for induction of Egr-1, and GSK3 β , which modulates the activity of the p65 subunit of NF- κ B.

These inhibitor studies demonstrated that many intracellular signaling pathways and transcription factors are required for LPS induction of TF expression in monocytes (–Fig. 5).

Inhibition of LPS Induction of TF Expression in Monocytes by Anti-inflammatory Cytokines

The effect of anti-inflammatory cytokines (IL-4, IL-10, and IL-13) on LPS induction of TF expression in monocytes has been studied by several groups. All three anti-inflammatory cytokines blocked LPS induction of TF expression in monocytes.^{81–84} In 1996, Ernoffsson et al⁸⁵ compared the inhibitory activity of different anti-inflammatory cytokines and showed that IL-10 exhibited significantly higher inhibitory activity compared with IL-4 and IL-13.

Regulation of Monocyte TF Activity

TF can be present on the cell surface in an inactive (encrypted) state or an active (decrypted) state.⁸⁶ Cell activation and injury increase the amount of TF in the active state. There are several mechanisms that have been shown to regulate TF activity on the cell surface. First, when the negatively charged phospholipid PS is externalized to the outer leaflet of the plasma membrane, it interacts with TF and induces a conformational change that increases its activity. Second, thiol-disulfide exchange pathways regulate the formation of an allosteric disulfide bond in TF that increases its activity. Third, TF activity is negatively regulated by sphingomyelin (SM). Cellular activation leads to translocation of acid sphingomyelinase (ASMase) to the outer leaflet of the plasma membrane where it degrades SM and increases TF activity. Another study found that

CD248 positively regulates TF activity.⁸⁷ CD248 is a type 1 transmembrane glycoprotein that has a cellular pattern of expression that is like TF. The human monocyte/macrophage cell line Mono Mac 6 expresses both TF and CD248. Knockdown of CD248 expression in Mono Mac 6 with siRNA reduced TF activity.

Protein disulfide isomerase (PDI) has been shown to regulate TF activity by altering the allosteric disulfide bond in TF. Bacitracin and quercetin-3-rutinoside (rutin) are two PDI inhibitors. A recent study showed that bacitracin and rutin inhibited LPS induction of TF protein expression and activity in CD14-positive human monocytes and human peripheral blood mononuclear cells (PBMCs), respectively.⁸⁸ In addition, rutin reduced LPS induction of TF mRNA in human PBMCs. Another PDI inhibitor PACMA-31 increased LPS induction of TF expression in PBMCs at low concentrations but reduced monocyte TF at high concentrations by converting it into a noncoagulant state.⁸⁹ Another study showed that inhibition of ASMase with the tricyclic antidepressant drugs desipramine and imipramine reduced LPS induction of TF activity on mouse PBMCs without affecting TF protein expression.⁹⁰ Similar results were observed with human monocyte-derived macrophages. These results suggest that LPS induction of TF activity in monocytes and macrophages requires induction of TF protein expression and decryption of TF by ASMase-dependent degradation of SM.

Role of TF in the Activation of Coagulation in Endotoxemia and Sepsis

In 1991, Taylor et al⁹¹ showed that administration of an anti-TF monoclonal antibody protected baboons in a LD100 *E. coli* model of septic shock (–Fig. 3). This study indicated that TF was a key effector molecule in the pathogenesis of sepsis. In 1994, Levi et al⁹² demonstrated that administration of an anti-TF monoclonal antibody to chimpanzees injected with LPS reduced the activation of coagulation measured thrombin–antithrombin complexes (TAT) and prothrombin activation fragment F1 + 2 (F1 + 2).

In 1996, three groups generated TF knockout mice^{93–95} (–Fig. 3). These mice did not survive to adulthood and therefore could not be used to study the effects of TF deficiency in different diseases. In 1998, we rescued TF knockout mice using a transgene that expresses low levels of human TF from the human TF promoter.⁹⁶ These so-called low TF mice could be used as a model to study the role of TF in different diseases. In 2004, we used low TF mice to examine the role of TF in a mouse model of endotoxemia⁹⁷ (–Fig. 3). We found that endotoxemic low TF mice exhibited a dramatic reduction in the activation of coagulation measured by TAT. Yang et al⁹⁸ also showed a reduction in TAT in low TF mice in a lethal endotoxemia model. In addition, they showed that TAT levels were significantly reduced in endotoxemic mice by the administration of a rat anti-mouse TF monoclonal antibody called 1H1. These studies demonstrate that TF mediated the activation of coagulation in animal models of endotoxemia and sepsis.

Role of Hematopoietic Cell, Myeloid, and Monocyte TF in the Activation of Coagulation in Endotoxemia and Sepsis

In 1983, Osterud and Flaegstad⁹⁹ showed that TF activity was increased in mononuclear cells from patients with meningococcal infection and proposed that this expression contributed to DIC. In 2000, Franco et al¹⁰⁰ analyzed TF mRNA expression in monocytes and activation of coagulation between 30 minutes and 24 hours in a human model of endotoxemia. They observed a transient increase in TF mRNA expression in monocytes with a maximal level at 3 hours and a return to baseline level by 24 hours. The activation of coagulation, which was assessed by measuring levels of TAT and F1 + 2, was maximal at 4 hours and returned to baseline at 24 hours. This study indicated that monocyte TF expression preceded the activation of coagulation, and it suggested that monocyte TF expression played a role in the activation of coagulation.

In 2004, we examined the role of hematopoietic cell TF in the activation of coagulation in a mouse endotoxemia model.⁹⁷ We transplanted bone marrow from low TF mice and wild-type mice into wild-type mice to determine the role of hematopoietic and nonhematopoietic cell TF in LPS activation of coagulation. Importantly, mice with bone marrow from low TF mice had significantly reduced activation of coagulation compared with mice with bone marrow from wild-type mice. In 2007, we generated TF-floxed mice¹⁰¹ (► Fig. 3). This allowed us to delete the TF gene in different cell types. We crossed TF-floxed mice with mice expressing the Cre recombinase expressed from the LysM promoter to delete the TF gene in myeloid cells.¹⁰² In 2010, we used these mice to determine the effect of deletion of the TF gene in myeloid cells on LPS activation of coagulation in mice.¹⁰² We found that deletion of TF in myeloid cells reduced the activation of coagulation by approximately 50%. These studies demonstrate that hematopoietic cell TF and myeloid cell TF play a major role in the activation of coagulation in a mouse model of endotoxemia.

LPS-Stimulated Monocytes Release TF-Positive Extracellular Vesicles

Activated cells, such as LPS-stimulated monocytes, release extracellular vesicles (EVs) into the blood.¹⁰³ In 1994, Satta et al¹⁰⁴ demonstrated that LPS stimulation of human monocytes for 5 hours induces the expression of TF and the release of EVs that contain TF (► Fig. 3). Other studies have also shown that LPS-stimulated monocytes release TF-positive EVs.^{39,48} We and others have shown that LPS stimulation of human whole blood for 5 hours leads to the generation of TF-positive EVs.^{105–108} It is presumed that these TF-positive EVs are derived from LPS-stimulated monocytes.

Levels of TF-Positive EVs Are Increased in Endotoxemia Models

In 2004, Aras et al¹⁰⁵ observed a transient increase in EV TF activity in the circulation in a human model of endotoxemia

with a maximal level at 4 hours (► Fig. 3). Another study used an updated EV TF activity assay to measure the response in healthy volunteers after administration of LPS.¹⁰⁹ Levels of monocytes, EV TF activity, and TAT were measured between 0 and 24 hours. Monocyte counts decreased at 30 minutes indicating activation and recruitment to tissues and this preceded an increase in EV TF activity, which was maximal at 4 hours. Levels of TAT showed a similar peak after administration of LPS. Another study observed a 10-fold increase in EV TF activity at 3 hours compared with 0 hours in a human model of endotoxemia.¹¹⁰

In 2009, we showed that LPS increased levels of EV TF activity in mice.¹¹¹ More recently, Ansari et al⁸⁶ also showed increased levels of EV TF activity in endotoxemic mice. Furthermore, they immunoprecipitated the EVs with an anti-TF antibody and then analyzed the presence of other cell type specific markers by western blotting. As expected, TF-positive EVs expressed the monocyte marker CD14. In addition, these EVs also expressed α -smooth muscle cell actin and CD248, which are markers of perivascular cells. These data suggest that TF-positive EVs in endotoxemic mice are derived from activated monocytes as well as perivascular cells.

We found that patients with sepsis have higher levels of EV TF activity compared with healthy controls.¹¹² Higher levels of EV TF activity were also observed in patients with meningococcal septic shock compared with patients with meningococcal meningitis.¹¹³ Levels of EV TF activity were associated with levels of LPS in the plasma. In addition, septic patients with DIC had higher levels of EV TF activity compared with healthy controls.¹¹⁴ Importantly, EV TF activity correlated with the DIC score. Release of TF-positive EVs from activated monocytes would be expected to greatly enhance activation of the coagulation system in blood. These studies strongly suggest that monocyte-derived TF-positive EVs contribute the activation of coagulation in endotoxemia and sepsis.

Monocyte TF Expression and Thrombosis during Infection and Sterile Inflammation

As discussed above, the coagulation system is part of the host immune system. Formation of a clot in blood vessels can trigger an innate immune response that is referred to as thromboinflammation or immunothrombosis.⁵ This may occur in response to an infection or due to the release of DAMPs from damaged cells into the extracellular environment (sterile inflammation). Monocyte TF plays a central role in immunothrombosis, which facilitates the containment, recognition and destruction of pathogens, and removal of damaged cells. However, monocyte TF can contribute to thrombosis (particularly venous thrombosis). We and another group showed that deletion of TF in myeloid cells reduced venous thrombosis in a mouse model.^{34,115}

In 1984, Osterud and Due¹¹⁶ found that monocyte TF activity was increased on 1 day after surgery in patients with benign and malignant tumors (► Fig. 3). Similarly, Johnson et al¹¹⁷ found that TF activity in PBMCs increases 1 day after

total knee arthroplasty. This monocyte TF expression precedes the time for venous thrombosis, which has been shown to occur 7 days postsurgery in a large epidemiology study.¹¹⁸ These studies suggest that monocyte TF contributes to venous thrombosis after surgery.

Inflammasomes and LPS-Induced Activation of Coagulation in Mice

Inflammasomes are cytosolic multiprotein complexes that sense PAMPs and DAMPs and activate caspases.¹¹⁹ Monocytes and macrophages contain inflammasomes. Canonical inflammasomes lead to the activation of caspase 1 whereas noncanonical inflammasomes lead to the activation of caspase 11 (►Fig. 6). Different agents, such as the TLR3 ligand polyinosinic:polycytidylic acid (poly I:C), are used for priming. This results in expression of inflammasome components and activation of pathways that facilitate increased uptake of extracellular LPS and other agents that can activate intracellular receptors independently of TLR4. The NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome is the most studied inflammasome and consists of a sensor (NLRP3), an adaptor (apoptosis-associated speck-like protein

containing a caspase recruitment domain, ASC), and an effector (caspase 1).¹²⁰ Cytosolic LPS is sensed by caspase 11 in mice and caspase 4 and 5 in humans and this induces noncanonical NLRP3 inflammasome activation.¹²⁰ There are several steps in the assembly of the NLRP3 inflammasome. First, NLRP3 oligomerizes and this recruits ASC. Next, pro-caspase 1 is recruited and undergoes self-cleavage and activation. The NLRP3 inflammasome cleaves pro-IL-1 β and pro-IL-18 to active IL-1 β and IL-18 that are then released from the cell. In addition, caspase 1 activates gasdermin D (GSDMD), which leads to the formation of nanopores and pyroptosis, which is a form of programmed cell death. There are several recent reviews on the role of inflammasomes in the activation of coagulation in sepsis.^{121–123} Therefore, in this review we will only discuss studies that analyze TF expression.

Several groups have analyzed the role of inflammasomes in the induction of TF expression in macrophages and in mice (►Table 2). Yang et al⁹⁸ determined the roles of TLR4 and caspase 11 in the activation of coagulation in a mouse model of endotoxemia. LPS increased plasma TAT levels and fibrin deposition in the liver and lung. Importantly, LPS-induced activation of coagulation was attenuated by approximately

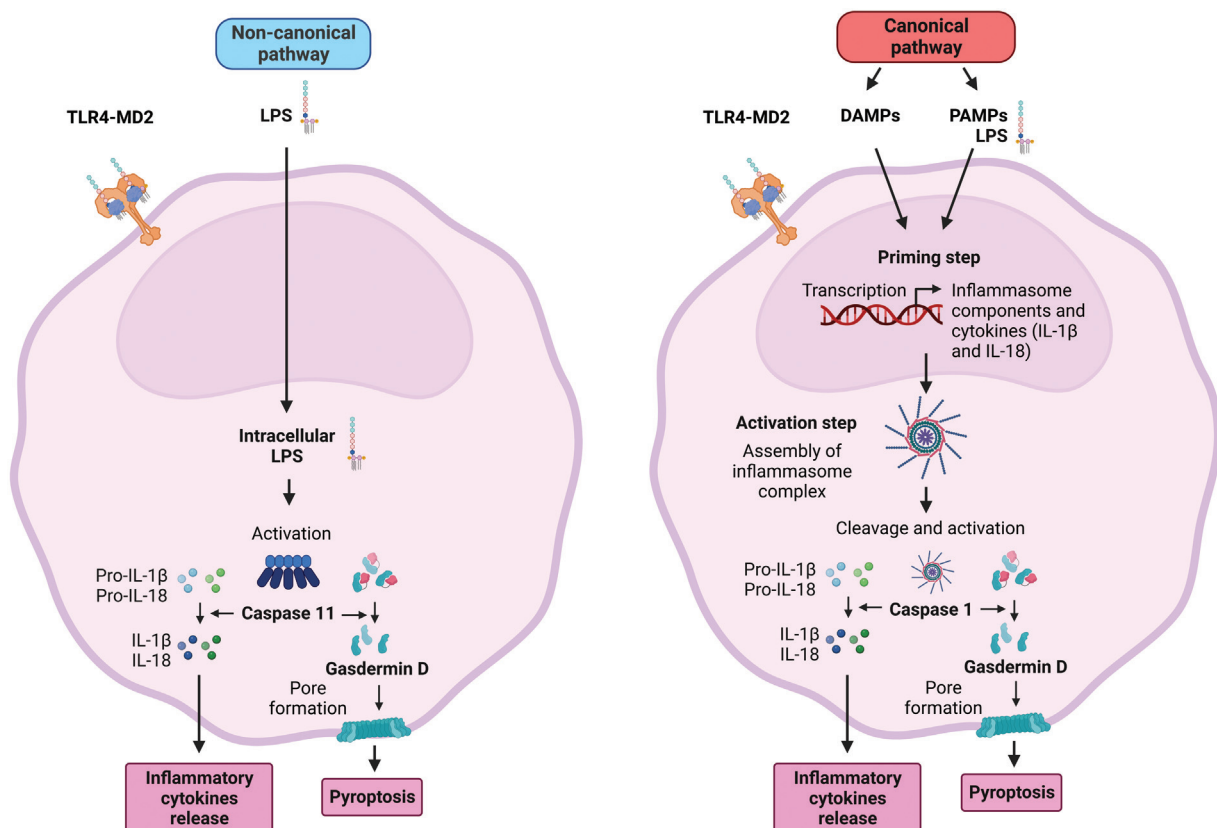


Fig. 6 Mechanisms of inflammasome activation. Inflammasomes are activated by the recognition of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Inflammasomes are divided into noncanonical and canonical pathways. Intracellular lipopolysaccharide (LPS) activates caspase 11 in the noncanonical inflammasome pathway. The canonical pathway consists of a priming step that leads to the transcription of inflammasome components and cytokines and an activation step that includes the assembly of the inflammasome and activation of caspase 1. Both inflammasome pathways cleave pro-IL-1 β , pro-IL-18, and gasdermin-D. The cleavage of interleukins induces the release of cytokines and the cleavage of gasdermin-D induces the formation of pores in the membranes, leading to a cell death via pyroptosis. Activation of inflammasomes is independent of the Toll-like receptor 4 (TLR4)–MD2 complex.

Table 2 Summary of studies that analyzed the role of inflammasomes in the induction of tissue factor expression in mouse macrophages and in mice

Model	Observations	Reference
Mice—LPS (10 mg/kg i.p.) for 8 hours	LPS increase in TAT levels is dependent on TLR4 and caspase 11	98
Mice—LPS (20 mg/kg i.p.) for 8 hours	LPS increase in TAT levels is dependent on ASC, NLRP3, and caspase 11	126
Mice—LPS (0.4 mg/kg i.p.) for 7 hours + LPS (10 mg/kg i.p.) for 8 hours	LPS increase in TAT levels is dependent on caspase 11 and GSDMD	98
Mice—poly I:C (10 mg/kg i.p.) for 7 hours + LPS (10 mg/kg i.p.) for 8 hours	LPS increase in TAT levels is dependent on caspase 11 but not TLR4	98
Mice—poly I:C (4 mg/kg i.p.) for 8 hours + LPS (50 mg/kg i.p.) for 4 hours	LPS increase in TAT levels is dependent on caspase 11 and GSDMD but not TLR4	127
Mice—EprJ for 90 minutes	LPS increase in TAT levels is dependent on caspase 1 and GSDMD but not TLR4	127
BMDMs—Poly I:C (1 µg/mL) for 5 hours + LPS (2 µg/mL) transfected	LPS induction of TF protein expression is dependent on caspase 11 and GSDMD but not TLR4	127
BMDMs—EprJ (100 ng/mL)	LPS induction of TF protein expression is dependent on caspase 1 and GSDMD but not TLR4	127
PMs—LPS (1 µg/mL) + cholera toxin subunit B	LPS induction of TF mRNA and protein expression is not dependent on GSDMD	98
PMs—LPS (1 µg/mL) + cholera toxin subunit B	LPS induction of TF activity is dependent on caspase 11, GSDMD, TMEM16F, and PS exposure	98

Abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; BMDMs, bone marrow-derived macrophages; GSDMD, gasdermin D; LPS, lipopolysaccharide; NLRP3, NOD-, LRR-, and pyrin domain-containing protein 3; PMs, peritoneal macrophages; poly I:C, polyinosinic:polycytidylic acid; PS, phosphatidylserine; TAT, thrombin–antithrombin complexes; TF, tissue factor; TLR4, Toll-like receptor 4, TMEM16F, transmembrane protein 16F.

50% in *Tlr4*-deficient mice and *Casp11*-deficient mice.⁹⁸ Similar results were observed with *E. coli*. Plasma TF activity was also reduced in endotoxemic *Casp11*-deficient mice and *Gsdmd*-deficient mice. Caspase 11 cleaves GSDMD as well as pannexin-1, which leads to activation of the purinergic receptor P2X7.¹²⁴ Interestingly, deletion of *Gsdmd* but not *P2x7r* reduced LPS-induced activation of coagulation.⁹⁸ Activation of coagulation in endotoxemic mice was also attenuated by administration of two PS binding proteins (lactadherin or milk fat globule-epidermal growth factor E8 [MFG-E8]), which indicated that PS was required for LPS-induced activation of coagulation in mice.⁹⁸ However, it should be noted that these PS-binding proteins have a general inhibitory effect on coagulation not just a selective inhibition of TF activity. Another study showed that caspase 11 and GSDMD were required for induction of TF in bone marrow-derived macrophages (BMDMs) and in mice exposed to outer membrane vesicles from *E. coli*.¹²⁵

A more recent study examined the role of the NLRP3 inflammasome in LPS-induced activation of coagulation in mice.¹²⁶ The dose of LPS (20 mg/kg) was higher than the previous study (→ **Table 2**). They found that a deficiency in either ASC, NLRP3, or caspase 11 decreased the level of TAT in endotoxemic mice by approximately 50% compared wild-type mice. Furthermore, the NLRP3 inhibitor MCC950 produced a similar decrease in the level of TAT in wild-type mice.¹²⁶ We also observed that MCC950 reduced TAT levels

in endotoxemic mice (Sachetto and Mackman, 2023, unpublished data). Levels of TF antigen in the plasma were decreased in mice deficient in ASC and NLRP3 but not caspase 11. In addition, MCC950 reduced the level of TF antigen in endotoxemic mice.

Wu et al¹²⁷ used the TLR3 ligand poly I:C to prime mice for 8 hours followed by stimulation with LPS for 4 hours to activate caspase 11. They found that the LPS-induced increase in plasma TAT was dependent on caspase 11 and GSDMD and independent of TLR4. Depletion of monocytes and macrophages with clodronate-containing liposome increased the survival of mice exposed to poly I:C and LPS. Yang et al⁹⁸ used a similar model to activated caspase 11 in a TLR4-independent manner by priming mice with poly I:C for 7 hours and stimulating with LPS for 8 hours. They also found that LPS-induced TAT in the plasma and fibrin deposition in tissues were reduced in *Casp11*^{-/-} but not *Tlr4*^{-/-} mice.

Wu et al¹²⁷ also analyzed the mechanism by which activation of caspase 1 leads to induction of TF expression in mice. They used the *E. coli* T3SS rod protein EprJ to activate caspase 1. Injection of EprJ into mice increased EV TF activity and TAT in plasma and fibrin deposition in the liver and spleen at 90 minutes. The EprJ-dependent increase in EV TF activity and TAT required caspase 1 and GSDMD but not TLR4. The EprJ-dependent increase in EV TF activity was also reduced in *Gsdmd*^{-/-} mice. Importantly, administration of

the rat anti-mouse TF monoclonal antibody 1H1 reduced levels of TAT and increased survival of the mice. In addition, treatment of mice with clodronate-containing liposomes to deplete monocytes and macrophages reduced levels of TAT and increased survival.

Wu et al¹²⁷ examined LPS induction of TF expression in BMDMs. Cells were primed with poly I:C and then transfected with LPS and incubated for 5 hours. LPS induced TF protein expression in primed cells and the release of TF-positive EVs into the culture supernatant. TF expression was dependent on caspase 11 and GSDMD and independent of TLR4. In addition, Wu et al¹²⁷ found that treatment of BMDMs with EprJ for 45 minutes induced TF protein expression and release of TF-positive EVs into the culture supernatant. The induction of TF protein expression was dependent on caspase 1 and GSDMD and independent of TLR4.¹²⁷

Yang et al⁹⁸ analyzed LPS induction of TF expression in mouse peritoneal macrophages (PMs). LPS was delivered into the cytosol of PMs using cholera toxin subunit B. LPS increased TF activity in the PMs, and this was dependent on caspase 11 and GSDMD. Lactadherin and MFG-E8 reduced TF activity of LPS-stimulated PMs. However, LPS induction of TF mRNA and protein expression in PMs was not reduced in *Gsdmd*^{-/-} PMs.⁹⁸ Externalization of PS is known to increase TF activity.¹²⁸ Yang et al⁹⁸ analyzed the mechanism of PS exposure and increased TF activity in PMs. They found that reduced expression of transmembrane protein 16F (TMEM16F), which is a calcium-dependent lipid scramblase, prevented LPS-induced PS exposure and TF activity. Finally, it was shown that LPS-induced PS exposure and TF activity was independent of pyroptosis.

Another study analyzed the role of transmembrane protein 173 (TMEM173, also known as STING) in the expression and release of TF in a mouse cecal ligation and puncture (CLP) model.¹²⁹ CLP induced DIC and fibrin deposition in lung, liver, and spleen. In addition, CLP increased levels of TF protein in the plasma measured using a commercial ELISA (Abcam, cat. # ab214091). Importantly, monocytes isolated from *Tmem173*^{-/-} mice 48 hours after CLP had reduced levels of TF protein in the culture supernatant but not TF mRNA in the cells compared with monocytes isolated from wild-type mice after CLP. This suggested that TMEM173 was required for the release of TF-positive EVs. Additional studies demonstrated that activation of TMEM173 led to an increase in intracellular Ca²⁺, activation of caspase 1, activation of GSDMD, and pyroptosis. Interestingly, *E. coli*-induced activation of GSDMD and TF release from BMDMs was abolished by double deletion of *Casp1* and *Casp11* but not by inhibition of caspase 8. In contrast, *Streptococcus pneumoniae*-induced activation of GSDMD and TF release from BMDMs was abolished by the caspase 8 inhibitor but not by a double deficiency of caspase 1 and 11. This indicates that different caspases activate GSDMD in response to different pathogens. ► **Fig. 7** shows a summary of the different studies.

It is difficult to compare these studies because they used different *in vitro* models, such as BMDMs and PMs, and different *in vivo* models. At present, it is unclear if TF expression by macrophages contributes to the activation of coagulation in the different models. As discussed above, we

have shown that hematopoietic cells and myeloid cells contribute to approximately 50% of the activation of coagulation in a mouse model of endotoxemia.^{97,102} In addition, increased vascular permeability, which occurs during endotoxemia and sepsis,^{130,131} will expose perivascular TF to blood and this will also contribute to the activation of coagulation. Clearly, additional studies are needed to resolve the different observations and to determine the cellular sources of TF that activates coagulation following activation of inflammasomes.

It should be noted that some of the assays used in these studies to measure TF may not be reliable. One study⁹⁸ used a commercial assay (AssayPro) to measure plasma TF activity. However, the commercial assays that claim to measure plasma TF activity do not distinguish between TF-dependent and TF-independent factor Xa generation.¹³² There are many different commercial ELISAs for both human and mouse TF. However, we found that four commercial ELISAs for human TF failed to detect TF in LPS-stimulated human whole blood.¹³³ Shi et al¹²⁶ used a mouse TF ELISA (ZC1B10, Cat# 2C-39071) to measure TF levels in plasma from endotoxemic mice but no controls were used to show the specificity of this ELISA. Two of the studies^{98,127} analyzed TF expression in macrophages using western blotting with an anti-TF antibody from Abcam (Cat# ab151748). We showed that this antibody does not recognize TF but rather binds to a nonspecific band that is present in TF-deficient cells.¹³⁴

Inflammasomes and Venous Thrombosis in Mice

Two studies analyzed the role of caspase 1 in a mouse model of inferior vena cava venous thrombosis. Zhang et al¹³⁵ found that *Casp1*^{-/-} mice and *Gsdmd*^{-/-} mice had smaller thrombi compared with wild-type mice. Thrombus size was also reduced in mice deficient in TF and in wild-type mice in which monocytes and macrophages had been depleted with gadolinium chloride. This treatment did not affect neutrophil counts. The authors concluded that TF from monocytes and macrophages plays a role in formation of the thrombus. However, mice with a selective deficiency of TF in monocytes and macrophages were not analyzed. Campos et al¹³⁶ showed that stimulation of neutrophils leads to the generation of active caspase 1 that is associated with neutrophil extracellular traps (NETs). Venous thrombi formed in the mouse inferior vena cava model also contained active caspase 1. Interestingly, a large amount of the caspase 1 was associated with platelets in the thrombus. Importantly, inhibition of caspase 1 reduced venous thrombosis in mice without affecting NET formation. These findings indicate that the canonical inflammasome pathway contributes to venous thrombosis in mice.

Monocyte TF Expression during Viral Infections

TF expression is increased in different cell types, including monocytes, during viral infections.¹³⁷ For instance, TF expression is increased in monocytes in people living with human immunodeficiency virus (HIV) compared with

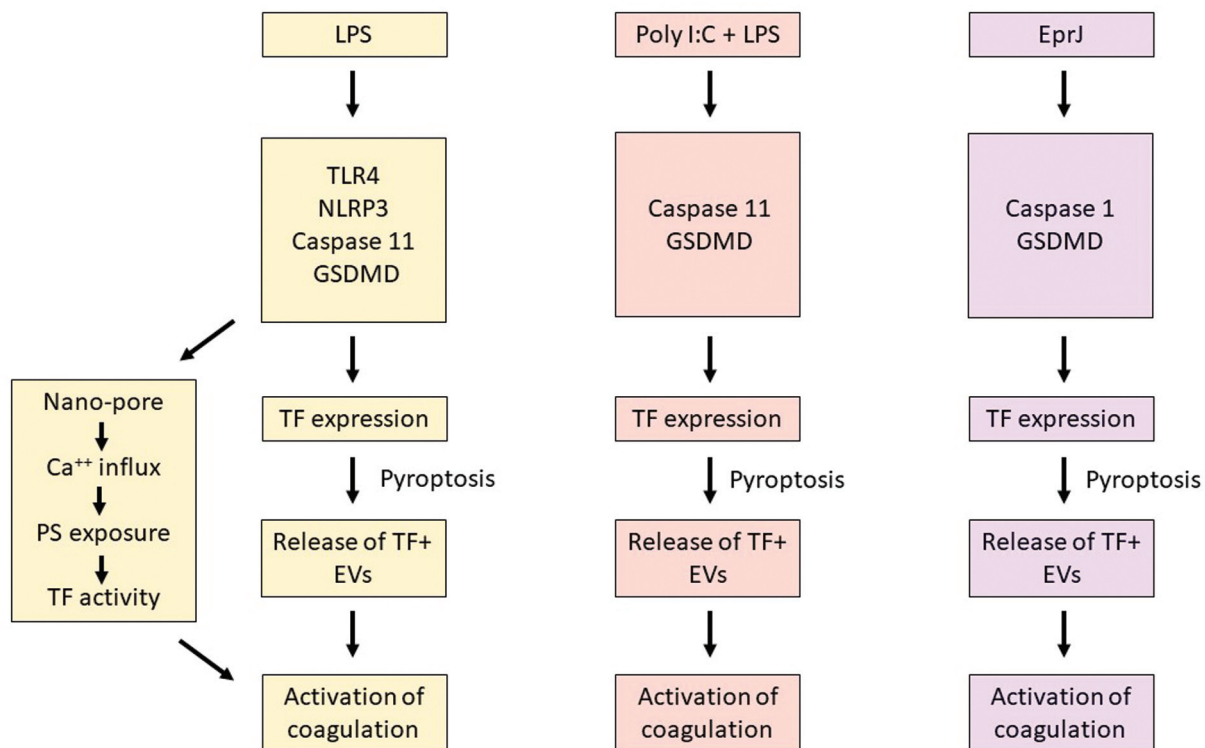


Fig. 7 Role of different inflammasomes in the induction of tissue factor (TF) expression and activation of coagulation. Lipopolysaccharide (LPS)-induced activation of coagulation was shown to be dependent on Toll-like receptor 4 (TLR4), the canonical inflammasome pathway (NOD-, LRR-, and pyrin domain-containing protein 3, [NLRP3]), the noncanonical inflammasome pathway (caspase 11), and gasdermin-D (GSDMD). These pathways lead to the expression of TF, release of TF-positive extracellular vesicles (EVs) after pyroptosis and activation of the coagulation. In addition, formation on nanopores in cell membranes leads to an increase in intracellular calcium, phosphatidylserine (PS) exposure, increased TF activity, and activation of coagulation. Priming with polyinosinic:polycytidylic acid (poly I:C) and stimulation with LPS induces the noncanonical inflammasome caspase 11/GSDMD pathway, whereas stimulation with bacterial rod protein EprJ induces the canonical caspase 1/GSDMD pathway. Both pathways lead to TF expression, release of TF-positive EVs after pyroptosis, and activation of coagulation.

healthy individuals.¹³⁸ Monocyte TF expression in people living with HIV also correlated with viral load and D-dimer.¹³⁹ Ebola virus induced TF mRNA expression in human PBMCs, and levels of TF mRNA were increased in PBMCs isolated from infected monkeys.¹⁴⁰ Monocytes in platelet-monocyte aggregates isolated from COVID-19 patients expressed TF.¹⁴¹ Another study showed that binding of platelets from COVID-19 patients to monocytes induces TF expression and this expression amplifies the expression of proinflammatory cytokines.¹⁴² Single-cell RNA sequencing of PBMCs from COVID-19 patients demonstrated a 5.2-fold increase in TF mRNA expression compared with controls.¹⁴³ Similar data were found using bulk RNA sequencing of sorted CD14-positive monocytes from COVID-19 patients. Levels of EV TF activity are increased in patients with COVID-19 and correlated with severity, thrombosis, and mortality.^{112,144–146} The cellular source of the TF-positive EVs has not been defined but depletion of leukocyte-derived EVs reduced the level of EV TF activity suggesting that activated monocytes contribute to the pool of circulating TF-positive EVs in COVID-19 patients.¹⁴⁷

Inhibition of the TF–FVIIa complex with the tick salivary anticoagulant protein Ixolaris reduced D-dimer levels, inflammation, immune activation, and plasma viremia in monkeys infected with simian immunodeficiency virus.¹³⁸ In addition,

inhibition of the TF–FVIIa complex using recombinant nematode anticoagulant protein c2 reduced mortality of monkeys infected with Ebola virus.¹⁴⁸ These studies suggest that TF, including monocyte TF expression, plays a central role in the coagulopathy and pathology associated with viral infections.

Conclusion

LPS induces transient expression of TF in monocytes that is part of the innate immune response to pathogens. However, monocyte TF can also induce thrombosis and DIC. Understanding the mechanisms that lead to the induction of TF expression in monocytes will allow the development of selective inhibitors to reduce pathologic activation of coagulation in endotoxemia and sepsis and other diseases without affecting hemostasis. A recent review described the induction of TF expression in monocytes by antiphospholipid antibodies.¹⁴⁹

Authors' Contribution

A.T.A.S. and N.M. wrote and edited the manuscript. All the authors read and approved the final manuscript.

Funding

This work was supported by the NIH NHLBI R35HL155657 (N.M) and the John C. Parker professorship (N.M).

Conflict of Interest

None declared.

Acknowledgment

Figures were made using BioRender.

References

- Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent J-L. Sepsis and septic shock. *Nat Rev Dis Primers* 2016;2(01):16045
- Amarante-Mendes GP, Adjemian S, Branco LM, Zanetti LC, Weinlich R, Bortoluci KR. Pattern recognition receptors and the host cell death molecular machinery. *Front Immunol* 2018; 9:2379
- Loof TG, Schmidt O, Herwald H, Theopold U. Coagulation systems of invertebrates and vertebrates and their roles in innate immunity: the same side of two coins? *J Innate Immun* 2011;3(01): 34–40
- Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine* 2008;42(02):145–151
- Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013;13(01):34–45
- Kawabata S, Muta T, Sadaaki Iwanaga: discovery of the lipopolysaccharide- and beta-1,3-D-glucan-mediated proteolytic cascade and unique proteins in invertebrate immunity. *J Biochem* 2010;147(05):611–618
- Howell W. Observations on the Chemical Composition and Coagulation of the Blood of *Limulus polyphemus*. *John Hopkins Univ Circular V*;1885;5:4–5
- Levin J, Bang FB. Clottable protein in *Limulus*; its localization and kinetics of its coagulation by endotoxin. *Thromb Diath Haemorrh* 1968;19(01):186–197
- Levin J, Bang FB. The role of endotoxin in the extracellular coagulation of *Limulus* blood. *Bull Johns Hopkins Hosp* 1964; 115:265–274
- Iwanaga S. The *limulus* clotting reaction. *Curr Opin Immunol* 1993;5(01):74–82
- Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science* 1964;145(3638):1310–1312
- MacFarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature* 1964; 202(4931):498–499
- Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 1989;134(05): 1087–1097
- Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol* 2018;38(04):709–725
- Mathison JC, Tobias PS, Wolfson E, Ulevitch RJ. Plasma lipopolysaccharide (LPS)-binding protein. A key component in macrophage recognition of gram-negative LPS. *J Immunol* 1992;149(01):200–206
- Tobias PS, Ulevitch RJ. Lipopolysaccharide binding protein and CD14 in LPS dependent macrophage activation. *Immunobiology* 1993;187(3–5):227–232
- Dunzendorfer S, Lee HK, Soldau K, Tobias PS. TLR4 is the signaling but not the lipopolysaccharide uptake receptor. *J Immunol* 2004; 173(02):1166–1170
- da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001;276(24):21129–21135
- Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995;13(01):437–457
- 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(5396):2085–2088
- Park BS, Lee J-O. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med* 2013;45(12):e66–e66
- World Health Organization Sepsis. World Health Organization. Accessed November 21, 2018 at: <http://www.who.int/news-room/fact-sheets/detail/sepsis>
- Gando S, Levi M, Toh C-H. Disseminated intravascular coagulation. *Nat Rev Dis Primers* 2016;2(01):16037
- Lerner RG, Goldstein R, Cummings G, Lange K. Stimulation of human leukocyte thromboplastic activity by endotoxin. *Proc Soc Exp Biol Med* 1971;138(01):145–148
- Rivers RPA, Hathaway WE, Weston WL. The endotoxin-induced coagulant activity of human monocytes. *Br J Haematol* 1975;30(03):311–316
- Semeraro N, Biondi A, Lorenzet R, Locati D, Mantovani A, Donati MB. Direct induction of tissue factor synthesis by endotoxin in human macrophages from diverse anatomical sites. *Immunology* 1983;50(04):529–535
- Mészáros K, Aberle S, Dedrick R, et al. Monocyte tissue factor induction by lipopolysaccharide (LPS): dependence on LPS-binding protein and CD14, and inhibition by a recombinant fragment of bactericidal/permeability-increasing protein. *Blood* 1994;83(09):2516–2525
- Steinemann S, Ulevitch RJ, Mackman N. Role of the lipopolysaccharide (LPS)-binding protein/CD14 pathway in LPS induction of tissue factor expression in monocytic cells. *Arterioscler Thromb* 1994;14(07):1202–1209
- Halvorsen H, Olsen JO, Osterud B. Granulocytes enhance LPS-induced tissue factor activity in monocytes via an interaction with platelets. *J Leukoc Biol* 1993;54(04):275–282
- Osterud B. Platelet activating factor enhancement of lipopolysaccharide-induced tissue factor activity in monocytes: requirement of platelets and granulocytes. *J Leukoc Biol* 1992;51(05): 462–465
- Engstad CS, Lia K, Rekdal O, Olsen JO, Osterud B. A novel biological effect of platelet factor 4 (PF4): enhancement of LPS-induced tissue factor activity in monocytes. *J Leukoc Biol* 1995;58(05):575–581
- Herbert JM, Corseaux D, Lale A, Bernat A. Hypoxia primes endotoxin-induced tissue factor expression in human monocytes and endothelial cells by a PAF-dependent mechanism. *J Cell Physiol* 1996;169(02):290–299
- Landsem A, Fure H, Christiansen D, et al. The key roles of complement and tissue factor in *Escherichia coli*-induced coagulation in human whole blood. *Clin Exp Immunol* 2015;182(01): 81–89
- Subramaniam S, Jurk K, Hobohm L, et al. Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. *Blood* 2017;129(16): 2291–2302
- Weinstein JR, Swarts S, Bishop C, Hanisch U-K, Möller T. Lipopolysaccharide is a frequent and significant contaminant in microglia-activating factors. *Glia* 2008;56(01):16–26
- Neumann F-J, Ott I, Marx N, et al. Effect of human recombinant interleukin-6 and interleukin-8 on monocyte procoagulant activity. *Arterioscler Thromb Vasc Biol* 1997;17(12):3399–3405
- Broze GJ Jr, Leykam JE, Schwartz BD, Miletich JP. Purification of human brain tissue factor. *J Biol Chem* 1985;260(20):10917–10920
- Carson SD, Ross SE, Bach R, Guha A. An inhibitory monoclonal antibody against human tissue factor. *Blood* 1987;70(02):490–493
- Basavaraj MG, Olsen JO, Østerud B, Hansen J-B. Differential ability of tissue factor antibody clones on detection of tissue factor in blood cells and microparticles. *Thromb Res* 2012;130(03):538–546

- 40 Hisada Y, Alexander W, Kasthuri R, et al. Measurement of microparticle tissue factor activity in clinical samples: a summary of two tissue factor-dependent FXa generation assays. *Thromb Res* 2016;139(139):90–97
- 41 Morrissey JH, Fair DS, Edgington TS. Monoclonal antibody analysis of purified and cell-associated tissue factor. *Thromb Res* 1988;52(03):247–261
- 42 Albrecht S, Luther T, Grossmann H, Flössel C, Kotsch M, Müller M. An ELISA for tissue factor using monoclonal antibodies. *Blood Coagul Fibrinolysis* 1992;3(03):263–270
- 43 Kirchhofer D, Moran P, Chiang N, et al. Epitope location on tissue factor determines the anticoagulant potency of monoclonal anti-tissue factor antibodies. *Thromb Haemost* 2000;84(06):1072–1081
- 44 Morrissey JH, Fakhrai H, Edgington TS. Molecular cloning of the cDNA for tissue factor, the cellular receptor for the initiation of the coagulation protease cascade. *Cell* 1987;50(01):129–135
- 45 Magdolen V, Albrecht S, Kotsch M, et al. Immunological and functional analyses of the extracellular domain of human tissue factor. *Biol Chem* 1998;379(02):157–165
- 46 Morrissey J, Agis H, Albrecht S, et al. CD142 (Tissue Factor) Workshop Panel report. In: Kishimoto et al, eds. *Leucocyte Typing VI: White Cell Differentiation Antigens*. New York, NY: Garland Publishing, Inc.; 1997:742–746
- 47 Huang M, Syed R, Stura EA, et al. The mechanism of an inhibitory antibody on TF-initiated blood coagulation revealed by the crystal structures of human tissue factor, Fab 5G9 and TF.G9 complex. *J Mol Biol* 1998;275(05):873–894
- 48 Egorina EM, Sovershaev MA, Bjørkøy G, et al. Intracellular and surface distribution of monocyte tissue factor: application to intersubject variability. *Arterioscler Thromb Vasc Biol* 2005;25(07):1493–1498
- 49 Spicer EK, Horton R, Bloem L, et al. Isolation of cDNA clones coding for human tissue factor: primary structure of the protein and cDNA. *Proc Natl Acad Sci U S A* 1987;84(15):5148–5152
- 50 Scarpati EM, Wen D, Broze GJ Jr, et al. Human tissue factor: cDNA sequence and chromosome localization of the gene. *Biochemistry* 1987;26(17):5234–5238
- 51 Mackman N, Morrissey JH, Fowler B, Edgington TS. Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade. *Biochemistry* 1989;28(04):1755–1762
- 52 Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. *Mol Cell Biol* 1989;9(06):2752–2755
- 53 Ollivier V, Houssaye S, Ternisien C, et al. Endotoxin-induced tissue factor messenger RNA in human monocytes is negatively regulated by a cyclic AMP-dependent mechanism. *Blood* 1993;81(04):973–979
- 54 Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc Natl Acad Sci U S A* 1986;83(06):1670–1674
- 55 Mackman N, Brand K, Edgington TS. Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both activator protein 1 and nuclear factor kappa B binding sites. *J Exp Med* 1991;174(06):1517–1526
- 56 Oeth P, Parry GC, Mackman N. Regulation of the tissue factor gene in human monocytic cells. Role of AP-1, NF-kappa B/Rel, and Sp1 proteins in uninduced and lipopolysaccharide-induced expression. *Arterioscler Thromb Vasc Biol* 1997;17(02):365–374
- 57 Oeth PA, Parry GC, Kunsch C, Nantermet P, Rosen CA, Mackman N. Lipopolysaccharide induction of tissue factor gene expression in monocytic cells is mediated by binding of c-Rel/p65 heterodimers to a kappa B-like site. *Mol Cell Biol* 1994;14(06):3772–3781
- 58 Hall AJ, Vos HL, Bertina RM. Lipopolysaccharide induction of tissue factor in THP-1 cells involves Jun protein phosphorylation and nuclear factor kappaB nuclear translocation. *J Biol Chem* 1999;274(01):376–383
- 59 Guha M, O'Connell MA, Pawlinski R, et al. Lipopolysaccharide activation of the MEK-ERK1/2 pathway in human monocytic cells mediates tissue factor and tumor necrosis factor alpha expression by inducing Elk-1 phosphorylation and Egr-1 expression. *Blood* 2001;98(05):1429–1439
- 60 Group ER, Donovan-Peluso M. Lipopolysaccharide induction of THP-1 cells activates binding of c-Jun, Ets, and Egr-1 to the tissue factor promoter. *J Biol Chem* 1996;271(21):12423–12430
- 61 Napoleone E, di Santo A, Peri G, et al. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol* 2004;76(01):203–209
- 62 Ternisien C, Ramani M, Ollivier V, et al. Endotoxin-induced tissue factor in human monocytes is dependent upon protein kinase C activation. *Thromb Haemost* 1993;70(05):800–806
- 63 Ternisien C, Ollivier V, Khechai F, Ramani M, Hakim J, de Prost D. Protein tyrosine kinase activation is required for LPS and PMA induction of tissue factor mRNA in human blood monocytes. *Thromb Haemost* 1995;73(03):413–420
- 64 Polack B, Pernod G, Barro C, Doussière J. Role of oxygen radicals in tissue factor induction by endotoxin in blood monocytes. *Haemostasis* 1997;27(04):193–200
- 65 Luyendyk JP, Piper JD, Tencati M, et al. A novel class of antioxidants inhibit LPS induction of tissue factor by selective inhibition of the activation of ASK1 and MAP kinases. *Arterioscler Thromb Vasc Biol* 2007;27(08):1857–1863
- 66 Pendurthi UR, Williams JT, Rao LVM. Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells: a possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. *Arterioscler Thromb Vasc Biol* 1999;19(02):419–426
- 67 Mackman N. Protease inhibitors block lipopolysaccharide induction of tissue factor gene expression in human monocytic cells by preventing activation of c-Rel/p65 heterodimers. *J Biol Chem* 1994;269(42):26363–26367
- 68 Hölschermann H, Dürfeld F, Maus U, et al. Cyclosporine a inhibits tissue factor expression in monocytes/macrophages. *Blood* 1996;88(10):3837–3845
- 69 Oeth P, Mackman N. Salicylates inhibit lipopolysaccharide-induced transcriptional activation of the tissue factor gene in human monocytic cells. *Blood* 1995;86(11):4144–4152
- 70 Osnes LT, Foss KB, Jøø GB, et al. Acetylsalicylic acid and sodium salicylate inhibit LPS-induced NF-kappa B/c-Rel nuclear translocation, and synthesis of tissue factor (TF) and tumor necrosis factor alpha (TNF-alpha) in human monocytes. *Thromb Haemost* 1996;76(06):970–976
- 71 De Prost D, Ollivier V, Hakim J. Pentoxifylline inhibition of procoagulant activity generated by activated mononuclear phagocytes. *Mol Pharmacol* 1990;38(04):562–566
- 72 Brozna JP, Horan M, Carson SD. Dipyridamole inhibits O2- release and expression of tissue factor activity by peripheral blood monocytes stimulated with lipopolysaccharide. *Thromb Res* 1990;60(02):141–156
- 73 Crutchley DJ, Hirsh MJ. The stable prostacyclin analog, iloprost, and prostaglandin E1 inhibit monocyte procoagulant activity in vitro. *Blood* 1991;78(02):382–386
- 74 Ollivier V, Ternisien C, Vu T, Hakim J, de Prost D. Pentoxifylline inhibits the expression of tissue factor mRNA in endotoxin-activated human monocytes. *FEBS Lett* 1993;322(03):231–234
- 75 Ollivier V, Parry GCN, Cobb RR, de Prost D, Mackman N. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. *J Biol Chem* 1996;271(34):20828–20835

- 76 Ferro D, Basili S, Alessandri C, Cara D, Violi F. Inhibition of tissue-factor-mediated thrombin generation by simvastatin. *Atherosclerosis* 2000;149(01):111–116
- 77 Markle RA, Han J, Summers BD, et al. Pitavastatin alters the expression of thrombotic and fibrinolytic proteins in human vascular cells. *J Cell Biochem* 2003;90(01):23–32
- 78 Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol* 1997;17(02):265–272
- 79 Nagata K, Ishibashi T, Sakamoto T, et al. Rho/Rho-kinase is involved in the synthesis of tissue factor in human monocytes. *Atherosclerosis* 2002;163(01):39–47
- 80 Guha M, Mackman N. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. *J Biol Chem* 2002;277(35):32124–32132
- 81 Herbert JM, Savi P, Laplace MC, Lale A. IL-4 inhibits LPS-, IL-1 β - and TNF α -induced expression of tissue factor in endothelial cells and monocytes. *FEBS Lett* 1992;310(01):31–33
- 82 Herbert JM, Savi P, Laplace M-C, et al. IL-4 and IL-13 exhibit comparable abilities to reduce pyrogen-induced expression of procoagulant activity in endothelial cells and monocytes. *FEBS Lett* 1993;328(03):268–270
- 83 Pradier O, Gérard C, Delvaux A, et al. Interleukin-10 inhibits the induction of monocyte procoagulant activity by bacterial lipopolysaccharide. *Eur J Immunol* 1993;23(10):2700–2703
- 84 Ramani M, Ollivier V, Ternisien C, et al. Interleukin 4 prevents the induction of tissue factor mRNA in human monocytes in response to LPS or PMA stimulation. *Br J Haematol* 1993;85(03):462–468
- 85 Ernoffsson M, Tenno T, Siegbahn A. Inhibition of tissue factor surface expression in human peripheral blood monocytes exposed to cytokines. *Br J Haematol* 1996;95(02):249–257
- 86 Ansari SA, Pendurthi UR, Rao LVM. Role of cell surface lipids and thiol-disulphide exchange pathways in regulating the encryption and decryption of tissue factor. *Thromb Haemost* 2019;119(06):860–870
- 87 Kapopara PR, Safikhani NS, Huang JL, et al. CD248 enhances tissue factor procoagulant function, promoting arterial and venous thrombosis in mouse models. *J Thromb Haemost* 2021;19(08):1932–1947
- 88 Beckmann L, Rolling CC, Voigtländer M, et al. Bacitracin and rutin regulate tissue factor production in inflammatory monocytes and acute myeloid leukemia blasts. *Cancers (Basel)* 2021;13(16):3941
- 89 Beckmann L, Mäder J, Voigtlaender M, et al. Inhibition of protein disulfide isomerase with PACMA-31 regulates monocyte tissue factor through transcriptional and posttranscriptional mechanisms. *Thromb Res* 2022;220:48–59
- 90 Wang J, Pendurthi UR, Rao LVM. Acid sphingomyelinase plays a critical role in LPS- and cytokine-induced tissue factor procoagulant activity. *Blood* 2019;134(07):645–655
- 91 Taylor FB Jr, Chang A, Ruf W, et al. Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. *Circ Shock* 1991;33(03):127–134
- 92 Levi M, ten Cate H, Bauer KA, et al. Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. *J Clin Invest* 1994;93(01):114–120
- 93 Carmeliet P, Mackman N, Moons L, et al. Role of tissue factor in embryonic blood vessel development. *Nature* 1996;383(6595):73–75
- 94 Bugge TH, Xiao Q, Kombrinck KW, et al. Fatal embryonic bleeding events in mice lacking tissue factor, the cell-associated initiator of blood coagulation. *Proc Natl Acad Sci* 1996;93(13):6258–6263
- 95 Toomey JR, Kratzer KE, Lasky NM, Stanton JJ, Broze GJ Jr. Targeted disruption of the murine tissue factor gene results in embryonic lethality. *Blood* 1996;88(05):1583–1587
- 96 Parry GC, Erlich JH, Carmeliet P, Luther T, Mackman N. Low levels of tissue factor are compatible with development and hemostasis in mice. *J Clin Invest* 1998;101(03):560–569
- 97 Pawlinski R, Pedersen B, Schabbauder G, et al. Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. *Blood* 2004;103(04):1342–1347
- 98 Yang X, Cheng X, Tang Y, et al. Bacterial endotoxin activates the coagulation cascade through gasdermin D-dependent phosphatidylserine exposure. *Immunity* 2019;51(06):983.e6–996.e6
- 99 Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. *Thromb Haemost* 1983;49(01):5–7
- 100 Franco RF, de Jonge E, Dekkers PE, et al. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. *Blood* 2000;96(02):554–559
- 101 Pawlinski R, Tencati M, Holscher T, et al. Role of cardiac myocyte tissue factor in heart hemostasis. *J Thromb Haemost* 2007;5(08):1693–1700
- 102 Pawlinski R, Wang JG, Owens AP III, et al. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. *Blood* 2010;116(05):806–814
- 103 Davidson SM, Boulanger CM, Aikawa E, et al. Methods for the identification and characterization of extracellular vesicles in cardiovascular studies - from exosomes to microvesicles. *Cardiovasc Res* 2023;119(01):45–63
- 104 Satta N, Toti F, Feugeas O, et al. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. *J Immunol* 1994;153(07):3245–3255
- 105 Aras O, Shet A, Bach RR, et al. Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. *Blood* 2004;103(12):4545–4553
- 106 Lee RD, Barcel DA, Williams JC, et al. Pre-analytical and analytical variables affecting the measurement of plasma-derived microparticle tissue factor activity. *Thromb Res* 2012;129(01):80–85
- 107 Vallier L, Bouriche T, Bonifay A, et al. Increasing the sensitivity of the human microvesicle tissue factor activity assay. *Thromb Res* 2019;182(182):64–74
- 108 Franco C, Lacroix R, Vallier L, et al. A new hybrid immunocapture bioassay with improved reproducibility to measure tissue factor-dependent procoagulant activity of microvesicles from body fluids. *Thromb Res* 2020;196(196):414–424
- 109 Woei-A-Jin FJ, De Kruid MD, Garcia Rodriguez P, Osanto S, Bertina RM. Microparticles expressing tissue factor are concurrently released with markers of inflammation and coagulation during human endotoxemia. *J Thromb Haemost* 2012;10(06):1185–1188
- 110 Mooberry MJ, Bradford R, Hobl EL, Lin FC, Jilma B, Key NS. Procoagulant microparticles promote coagulation in a factor XI-dependent manner in human endotoxemia. *J Thromb Haemost* 2016;14(05):1031–1042
- 111 Wang J-G, Manly D, Kirchofer D, Pawlinski R, Mackman N. Levels of microparticle tissue factor activity correlate with coagulation activation in endotoxemic mice. *J Thromb Haemost* 2009;7(07):1092–1098
- 112 Campbell RA, Hisada Y, Denorme F, et al. Comparison of the coagulopathies associated with COVID-19 and sepsis. *Res Pract Thromb Haemost* 2021;5(04):e12525
- 113 Hellum M, Øvstebø R, Brusletto BS, Berg JP, Brandtzaeg P, Henriksson CE. Microparticle-associated tissue factor activity correlates with plasma levels of bacterial lipopolysaccharides in meningococcal septic shock. *Thromb Res* 2014;133(03):507–514
- 114 Meng S, Kang K, Fei D, et al. Preliminary study of microparticle coagulation properties in septic patients with disseminated intravascular coagulation. *J Int Med Res* 2021;49(05):3000605211014094

- 115 von Brühl M-L, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med* 2012;209(04):819–835
- 116 Osterud B, Due Jr. Blood coagulation in patients with benign and malignant tumours before and after surgery. Special reference to thromboplastin generation in monocytes. *Scand J Haematol* 1984;32(03):258–264
- 117 Johnson GJ, Leis LA, Bach RR. Tissue factor activity of blood mononuclear cells is increased after total knee arthroplasty. *Thromb Haemost* 2009;102(04):728–734
- 118 White RH, Romano PS, Zhou H, Rodrigo J, Bargar W. Incidence and time course of thromboembolic outcomes following total hip or knee arthroplasty. *Arch Intern Med* 1998;158(14):1525–1531
- 119 Schroder K, Tschopp J. The inflammasomes. *Cell* 2010;140(06):821–832
- 120 Swanson KV, Deng M, Ting JP-Y. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol* 2019;19(08):477–489
- 121 Wu R, Wang N, Comish PB, Tang D, Kang R. Inflammasome-dependent coagulation activation in sepsis. *Front Immunol* 2021;12:641750
- 122 Tang D, Wang H, Billiar TR, Kroemer G, Kang R. Emerging mechanisms of immunocoagulation in sepsis and septic shock. *Trends Immunol* 2021;42(06):508–522
- 123 Ryan TAJ, Preston RJS, O'Neill LAJ. Immunothrombosis and the molecular control of tissue factor by pyroptosis: prospects for new anticoagulants. *Biochem J* 2022;479(06):731–750
- 124 Yang D, He Y, Muñoz-Planillo R, Liu Q, Núñez G. Caspase-11 requires the pannexin-1 channel and the purinergic P2X7 pore to mediate pyroptosis and endotoxic shock. *Immunity* 2015;43(05):923–932
- 125 Peng Y, Gao M, Liu Y, et al. Bacterial outer membrane vesicles induce disseminated intravascular coagulation through the caspase-11-gasdermin D pathway. *Thromb Res* 2020;196:159–166
- 126 Shi J, Tang Y, Liang F, et al. NLRP3 inflammasome contributes to endotoxin-induced coagulation. *Thromb Res* 2022;214(214):8–15
- 127 Wu C, Lu W, Zhang Y, et al. Inflammasome activation triggers blood clotting and host death through pyroptosis. *Immunity* 2019;50(06):1401.e4–1411.e4
- 128 Rao LVM, Pendurthi UR. Regulation of tissue factor coagulant activity on cell surfaces. *J Thromb Haemost* 2012;10(11):2242–2253
- 129 Zhang H, Zeng L, Xie M, et al. TMEM173 drives lethal coagulation in sepsis. *Cell Host Microbe* 2020;27(04):556–570.e6
- 130 Fu P, Birukova AA, Xing J, et al. Amifostine reduces lung vascular permeability via suppression of inflammatory signalling. *Eur Respir J* 2009;33(03):612–624
- 131 Kataoka H, Ushiyama A, Akimoto Y, Matsubara S, Kawakami H, Iijima T. Structural behavior of the endothelial glycocalyx is associated with pathophysiological status in septic mice: an integrated approach to analyzing the behavior and function of the glycocalyx using both electron and fluorescence intravital microscopy. *Anesth Analg* 2017;125(03):874–883
- 132 Mackman N, Sachetto ATA, Hisada Y. Measurement of tissue factor-positive extracellular vesicles in plasma: strengths and weaknesses of current methods. *Curr Opin Hematol* 2022;29(05):266–274
- 133 Sachetto ATA, Archibald SJ, Bhatia R, Monroe D, Hisada Y, Mackman N. Evaluation of four commercial ELISAs to measure tissue factor in human plasma. *Res Pract Thromb Haemost* 2023;7(03):100133
- 134 Rosell A, Moser B, Hisada Y, et al. Evaluation of different commercial antibodies for their ability to detect human and mouse tissue factor by western blotting. *Res Pract Thromb Haemost* 2020;4(06):1013–1023
- 135 Zhang Y, Cui J, Zhang G, et al. Inflammasome activation promotes venous thrombosis through pyroptosis. *Blood Adv* 2021;5(12):2619–2623
- 136 Campos J, Ponomaryov T, De Prendergast A, et al. Neutrophil extracellular traps and inflammasomes cooperatively promote venous thrombosis in mice. *Blood Adv* 2021;5(09):2319–2324
- 137 Antoniak S, Mackman N. Coagulation, protease-activated receptors, and viral myocarditis. *J Cardiovasc Transl Res* 2014;7(02):203–211
- 138 Schechter ME, Andrade BB, He T, et al. Inflammatory monocytes expressing tissue factor drive SIV and HIV coagulopathy. *Sci Transl Med* 2017;9(405):eaam5441
- 139 Funderburg NT, Mayne E, Sieg SF, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood* 2010;115(02):161–167
- 140 Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis* 2003;188(11):1618–1629
- 141 Hottz ED, Martins-Gonçalves R, Palhinha L, et al. Platelet-monocyte interaction amplifies thromboinflammation through tissue factor signaling in COVID-19. *Blood Adv* 2022;6(17):5085–5099
- 142 Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood* 2020;136(11):1330–1341
- 143 Girard TJ, Antunes L, Zhang N, et al. Peripheral blood mononuclear cell tissue factor (F3 gene) transcript levels and circulating extracellular vesicles are elevated in severe coronavirus 2019 (COVID-19) disease. *J Thromb Haemost* 2023;21(03):629–638
- 144 Rosell A, Havervall S, von Meijenföldt F, et al. Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality—brief report. *Arterioscler Thromb Vasc Biol* 2021;41(02):878–882
- 145 Guervilly C, Bonifay A, Burtsey S, et al. Dissemination of extreme levels of extracellular vesicles: tissue factor activity in patients with severe COVID-19. *Blood Adv* 2021;5(03):628–634
- 146 Francischetti IMB, Toomer K, Zhang Y, et al. Upregulation of pulmonary tissue factor, loss of thrombomodulin and immunothrombosis in SARS-CoV-2 infection. *EClinicalMedicine* 2021;39:101069
- 147 Mackman N, Grover SP, Antoniak S. Tissue factor expression, extracellular vesicles, and thrombosis after infection with the respiratory viruses influenza A virus and coronavirus. *J Thromb Haemost* 2021;19(11):2652–2658
- 148 Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 2003;362(9400):1953–1958
- 149 Salet DM, Bekkering S, Middeldorp S, Van DenHoogen LL. Targeting thromboinflammation in antiphospholipid syndrome. *J Thromb Haemost* 2023;21(04):744–757