Thromboinflammation as a Driver of Venous Thromboembolism

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Abstract

Thrombus formation has been identified as an integral part in innate immunity, termed immunothrombosis. Activation of host defense systems is known to result in a procoagulant environment. In this system, cellular players as well as soluble mediators interact with each other and their dysregulation can lead to the pathological process of thromboinflammation. These mechanisms have been under intensified investigation during the COVID-19 pandemic. In this review, we focus on the underlying mechanisms leading to thromboinflammation as one trigger of venous thromboembolism.

Keywords

- thromboinflammation
- immunothrombosis
- innate immunity
- venous thromboembolism
- COVID-19

Zusammenfassung

Die Thrombusbildung wurde als integraler Bestandteil der angeborenen Immunität identifiziert und als Immunthrombose bezeichnet. Es ist bekannt, dass die Aktivierung von Wirtsabwehrsystemen zu einer pro-thrombotischen Umgebung führt. In diesem System interagieren sowohl zelluläre Bestandteile als auch lösliche Faktoren miteinander, die bei einer Dysregulation den pathologischen Prozess der Thromboinflammation induzieren können. Diese Mechanismen wurden während der COVID-19-Pandemie verstärkt untersucht. In dieser Übersichtsarbeit konzentrieren wir uns auf die zugrunde liegenden Mechanismen, die zur Thromboinflammation führen als ein Auslöser der venösen Thromboembolien.

Schlüsselwörter

- Thromboinflammation
- Immunthrombose
- angeborene Immunität
- venöse Thromboembolien
- COVID-19

Introduction

Venous thromboembolism (VTE) with its manifestations deep vein thrombosis (DVT) and pulmonary embolism remains a major health care challenge.[1] Besides its obvious role in wound closure, thrombus formation has also been identified as an integral part in innate immunity, termed immunothrombosis.[2] Activation of host defense systems in response to invading pathogens is known to result in a procoagulant environment, which promotes thrombin generation. This cross-link between humoral and cellular amplification pathways as part of the physiological host defense (in this review defined as "immunothrombosis") should be differentiated from pathophysiological events during "thromboinflammation,"[3–5] where an overactivation of blood cells, coagulation system, and endothelial cells in response to pathogens or inflammatory triggers results in pathological thrombotic events (►Fig. 1).

Immunothrombosis mostly occurs in capillaries and venules without a major harm for the host to contain and neutralize foreign pathogens. However, when these mechanisms proceed uncontrolled, it can lead to pathological thrombosis, such as arterial thrombosis or DVT or disseminated intravascular coagulation in sepsis.[6,7] In this review,

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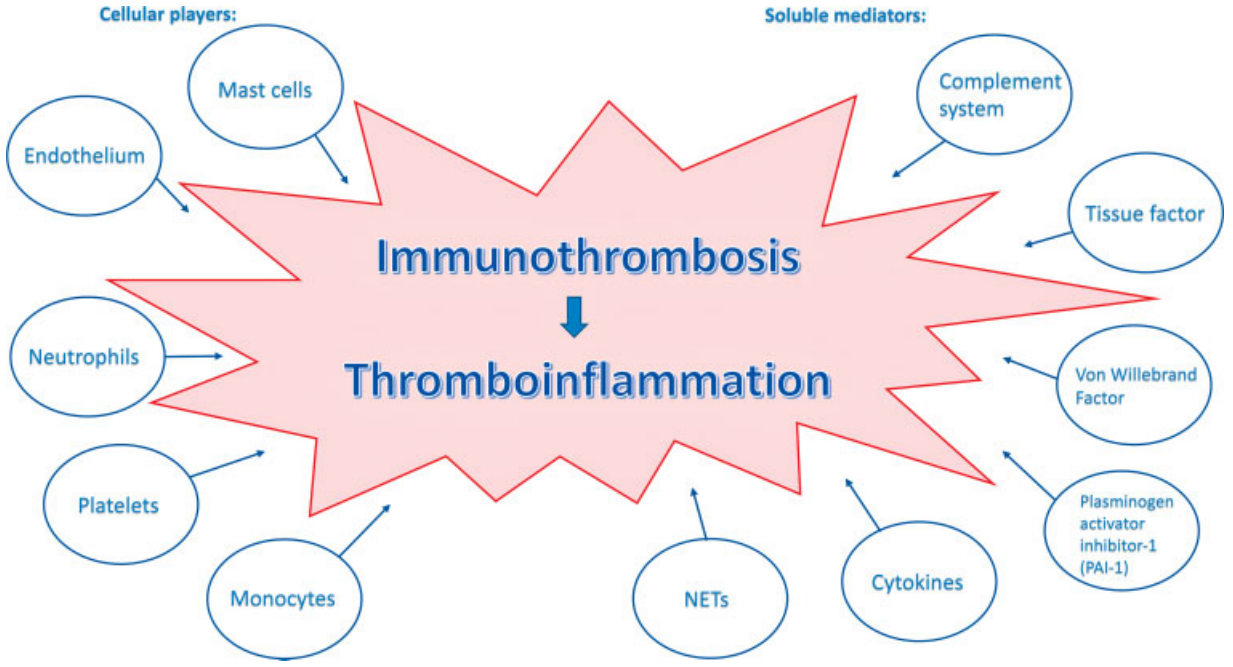

Fig. 1 Cellular players and soluble mediators driving immunothrombosis and thromboinflammation.

we focus on thromboinflammation as a driver of VTE and the novel insights into the underlying mechanisms.

Cellular Players and NETs

Mouse studies have revealed that monocytes, neutrophils, and platelets, in concert with the endothelium, are involved in the development of DVT.[6] Different triggers such as ischemic events, infections, or toxins can induce endothelial dysfunction.[8] For example, endothelial activation in endotoxemia culminates in augmented thrombus formation mediated through ICAM-1 (intercellular adhesion molecule-1) and TLR-1 (toll-like receptor 1).[9] In addition, direct pathogen interaction with endothelial cells can stimulate the release of different mediators.[10] Hypoxia is another pathological condition that leads to endothelial dysfunction characterized by increased permeability, a proinflammatory state, and decreased anticoagulant features.[11] Specifically, hypoxia induces upregulation of von Willebrand factor (VWF).[12] Mast cells located in the venous vessel wall release their mediators in response to reduced blood flow and further activate endothelium, which leads to Weibel–Palade body release.[13] Especially VWF release from Weibel–Palade bodies is crucial for deep vein thrombus formation by mediating platelet adhesion via glycoprotein Ibα (GPIbα).[14,15] Platelet adhesion leads to leukocyte recruitment to the vessel wall and thereby activating innate immunity.[5] Consistently, GPIbα-deficient mice showed impaired platelet and leukocyte accumulation along the endothelium.[6] In addition, platelets themselves sense infection and can be activated by pathogens. Consequently, they form aggregates, trigger the coagulation cascade, and recruit neutrophils and monocytes to prevent the spread of pathogens, thereby actively contributing to thromboinflammation.[16] Furthermore, clinical trials demonstrated that antiplatelet therapy is beneficial in preventing recurrent venous thromboembolic events.[17,18]

Leukocyte recruitment is essential for the development of DVT.[6,19] The activation of the coagulation system in venous thrombosis depends on blood-derived tissue factor (TF), which is mainly released by monocytes and locally activated by protein disulfide isomerase.[20,21] Moreover, neutrophil extracellular traps (NETs) particularly contribute to immunothrombosis by building a scaffold of chromatin and inflammatory and prothrombotic proteins and entrap cells, including activated platelets, enhancing thrombus formation as a positive feedback loop.[22–25] Direct interaction with pathogens or microbial components, cytokines, and complement factors can induce the release of NETs.[26] Notably, the formation of NETs is promoted by neutrophil interaction with activated platelets.[27] If NETosis is inhibited[28] or NETs are dissolved by DNase,[6,25] mice are protected from development of DVT in a stenosis model.

Addressing NETosis and cell recruitment in venous thrombosis promises new therapeutic targets in the prophylaxis and therapy of VTE.

Complement Factors and Cytokines

Innate immune cells are the main cellular drivers of immunothrombosis as described above. In addition, this process is molecularly regulated by the crosstalk between the coagulation cascade, the complement system, and the cytokines. The complement system can be activated via several pathways and several complement factors can activate platelets, neutrophils, induce endothelial secretion of VWF, and cause endothelial damage.[29–32] The complement system is an important host defense mechanism that involves a cascade

of processes, leading to the formation of the terminal membrane attack complex (MAC) C5b-9. MAC creates a transmembrane channel, triggering cell lysis and death when inserted into the cell membrane of an infected cell or directly onto a pathogen.[31] When these physiological defenses are hyperactivated, they result in excess endothelial damage that can serve as foci for thrombosis. Besides the endothelial damage caused by the complement system, which increases the thrombotic risk, the individual complement components are prothrombotic. Complement component 5a (C5a), for instance, can upregulate the activity of TF and plasminogen activator inhibitor-1 (PAI-1) and can activate neutrophils, resulting in promotion in the formation of NETs.[31] This corresponds to the findings seen in a mouse model where susceptibility to DVT strongly correlates with C5a levels,[33] as well as in humans where high levels of the C3 are associated with a high risk of DVT.[34]

Cytokines on the other hand, which are proteins secreted by various cells including immune cells, serve as an important innate defense mechanism as they recruit adaptive immune cells, and regulate a wide range of processes in the immune system.[35] Cytokines have prothrombotic effects, such as interleukin-6 which increases platelet production and activity, increases the expression of TF on endothelial cells and monocytes, and can also give rise to endothelial dysfunction.[36,37] Interferon-γ similarly increases platelet production and impairs the vascular endothelium, which in turn increases prothrombotic effects.[37] Interleukin-2 upregulates PAI-1 which can decrease fibrinolysis.[37] It is important to note that not only inflammation causes thrombosis but thrombosis can in turn directly trigger inflammation and a tight, bidirectional connection exists between inflammation and thrombosis.

Intervening in these cross-talks promises future therapeutic options.

COVID-19

During the coronavirus disease 2019 (COVID-19) pandemic, increased incidences of thrombotic complications have been observed.[38] The mechanisms contributing to increased thrombosis in COVID-19 involve extensive cross-talk between hemostasis and the immune system.[39] In COVID-19 there are two entities leading to immunothrombosis and thromboinflammation.

One is local immunothrombosis in pulmonary vessels mediated by the infection of alveolar epithelium with the pathogen SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) via the ACE2 (angiotensin converting enzyme 2) receptor. This leads to a release of inflammatory cytokines such as interleukin-6 and tumor necrosis factor and chemokines such as interleukin-8 and CCL (chemokine [C–C motif] ligand)2 and CCL3[40,41] which thereby activate epithelial cells, monocytes, and neutrophils. Endothelial cells themselves can also be infected by SARS-CoV-2 via the ACE2 receptor leading to activation and dysfunction. This leads to the activation of the coagulation system and by activating platelets the proinflammatory state is furthermore triggered leading to local coagulation lesions.[42,43] Interleukin-6 levels show a correlation with fibrinogen levels in COVID-19 patients supporting the theory of thromboinflammation.[44]

In addition to the local immunothrombosis/thromboinflammation in COVID-19 patients, the infection can also lead to a systemic hypercoagulable state, leading to macro- and microvascular thrombosis as a result of thromboinflammation. The overactivation of the complement system leads to the activation of the alternative and lectin pathway which interacts with the coagulation pathway.[45,46] Furthermore SARS-CoV-2 stimulates the ACE2 receptor and thereby disrupts the renin–angiotensin system, which leads to vasoconstriction and proinflammatory cytokine release,[47] which can trigger a cytokine storm and a systemic inflammatory response. The systemic cytokine release activates endothelial cells in the whole organism leading to endothelial dysfunction.[48] Furthermore, the impact of NETs in COVID-19 has been extensively described to contribute to the procoagulant and proinflammatory state.[49] Autopsy studies revealed the occurrence of NETs in lungs from deceased COVID-19 patients.[50–52] Moreover, soluble indicators of NETs have been widely detected in the plasma and sera of COVID-19 patients.[53] Most importantly, NETs in cooperation with TF and the complement system were associated with thrombotic events.[54–56]

Increased levels of antiphospholipid antibodies have been detected in critically ill patients with COVID-19.[57,58] In the antiphospholipid syndrome, these antibodies remain elevated over time and are known for the development of thromboembolic events. Their role in the development of thromboinflammation in COVID-19 remains controversial, as they are transiently elevated in many acute illnesses and the underlying mechanisms are not yet clearly understood.[59,60]

Also platelets have been proposed to be prothrombotic players in COVID-19.[61] The majority of studies found hyperactivated platelets during SARS-CoV-2 infection.

Several therapeutic intervention strategies to reduce the risk of developing thrombosis have been proposed to be useful in COVID-19 pathology.[62] They include direct targeting of the coagulation cascade, antiplatelet drugs, inhibitors of NET formation as well as complement and cytokine blockade. However, effective treatment options are still lacking.

Conclusion

The mechanisms leading to VTE are complex and closely linked to the innate immune system as well as to inflammatory processes (►Fig. 1). The most recent and prominent example for these close interactions is the current COVID-19 pandemic with its high incidences of thrombotic complications.

Unraveling these pathomechanisms promises future therapeutic strategies to prevent thromboembolic complications.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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