# Thrombosis as an intravascular effector of innate immunity

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Abstract | Thrombosis is the most frequent cause of mortality worldwide and is closely linked to haemostasis, which is the biological mechanism that stops bleeding after the injury of blood vessels. Indeed, both processes share the core pathways of blood coagulation and platelet activation. Here, we summarize recent work suggesting that thrombosis under certain circumstances has a major physiological role in immune defence, and we introduce the term immunothrombosis to describe this process. Immunothrombosis designates an innate immune response induced by the formation of thrombi inside blood vessels, in particular in microvessels. Immunothrombosis is supported by immune cells and by specific thrombosis-related molecules and generates an intravascular scaffold that facilitates the recognition, containment and destruction of pathogens, thereby protecting host integrity without inducing major collateral damage to the host. However, if uncontrolled, immunothrombosis is a major biological process fostering the pathologies associated with thrombosis.

Thrombosis
The formation of a thrombus (clot) inside blood vessels, resulting in partial or complete vessel occlusion.

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Haemostasis is an important biological process that prevents bleeding after vessel injury. It is mediated by two main mechanisms — blood coagulation and the activation of platelets. Thrombosis is generally considered to be a pathological deviation of haemostasis and also involves coagulation and platelet activation. Thrombosis is characterized by intravascular thrombus (clot) formation and vessel occlusion. Thrombotic processes may affect both arteries and veins and are the leading cause of death worldwide. In the United States, more than 2,000 patients die each day as a direct result of clot formation in coronary or cerebral arteries triggering myocardial infarction and stroke, respectively[1]. Likewise, deep vein thrombosis (DVT) is a major cause of morbidity and mortality owing to the development of pulmonary embolism, a life-threatening sequela that occurs when the thrombus breaks apart and travels to the lung, causing occlusion of a pulmonary artery[2]. Thrombosis is therefore a major healthcare issue, and the prevalence of its complications continues to rise at an alarming pace. Although effective antithrombotic approaches have been developed to combat this cardiovascular epidemic, current regimens target both pathological thrombosis and physiological haemostasis, and therefore almost inevitably increase the risk of bleeding. Thus, there is a need for the identification of novel strategies that allow for the specific inhibition of arterial and/or venous thrombosis without compromising normal haemostasis.

Arterial thrombosis and venous thrombosis are separate disease entities with distinct pathogenic features. Arterial thrombosis is predominantly triggered by atherosclerotic plaque rupture (a process referred to as atherothrombosis). By contrast, a frequent cause of venous thrombosis is blood stasis in the absence of endothelial injury[3–5]. Despite these differences, arterial thrombosis and venous thrombosis both involve the activation of platelets and the coagulation cascade (leading to the formation of fibrin), which are processes that are also core elements of normal haemostasis. However, emerging work suggests that arterial and venous thrombosis also involves distinct processes from haemostasis that occur mostly in the intravascular compartment. These 'thrombosis-related signatures' depend on the active participation of innate immune cells (particularly monocytes and neutrophils, as well as dendritic cells), which initiate and propagate fibrin formation and trigger platelet activation during the development of thrombosis.

Although the prothrombotic actions of innate immune cells are detrimental in the context of pathological thrombosis in larger arteries and veins, recent findings suggest that under certain circumstances thrombosis is a physiological process that constitutes an intrinsic effector mechanism of innate immunity. We refer to this process here as 'immunothrombosis'. Immunothrombosis is in part a consequence of the evolutionary conserved link between blood coagulation

Myocardial infarction
An episode of acute cardiac ischaemia that leads to the death of heart-muscle cells. It is usually caused by the rupture of an atherosclerotic plaque leading to clot formation.

Atherothrombosis
Following the rupture of unstable atherosclerotic plaques, thrombogenic material becomes exposed or released and triggers thrombus formation and eventually the occlusion of an artery.

Haemostasis
A biological process that repairs perforations in blood vessels via the formation of a thrombus consisting of aggregating platelets and fibrin in the vessel wall.

Disseminated intravascular coagulation
(DIC; also known as consumptive coagulopathy). A pathological process in which the blood begins to coagulate throughout the entire body. During this process, platelets and coagulation factors are depleted, resulting in a paradoxical situation in which there is a high risk of simultaneous fatal thrombosis and large-scale haemorrhage. DIC often occurs in critically ill patients with overwhelming infection, fulminant sepsis, extensive tissue damage or malignancy.

and innate immunity[6–10] (BOX 1). Immunothrombosis is increasingly recognized as an independent line of host defence that is supported by specific molecular mechanisms. It mediates the recognition of pathogens and damaged cells, and inhibits pathogen dissemination and survival. Immunothrombosis can therefore be regarded as a newly identified, crucial element of intravascular immunity, which is a part of the immune system that encompasses a wide range of host strategies to detect and protect against pathogens in the vasculature[11]. However, dysregulation of immunothrombosis is likely to constitute a key event in the development of thrombotic disorders, including myocardial infarction, stroke and disseminated intravascular coagulation (DIC). As some of the molecular mediators of immunothrombosis are distinct and at least partly dispensable for normal haemostasis, targeting immunothrombosis rather than haemostasis could provide new options to treat and prevent pathological thrombosis.

In this Review, we summarize our current knowledge regarding the key molecular and cellular mechanisms that mediate immunothrombosis and outline their potential physiological roles in host defence. We also describe how the dysregulation of immunothrombosis might contribute to pathological blood vessel obstructions and examine the therapeutic implications of dissecting immunothrombosis mechanisms.

## Traditional players of haemostasis

The mammalian blood system depends on a closed, high-pressure vascular circuit in order to fulfil its multiple biological functions, including oxygen delivery and host defence against pathogen intruders. When the vessel wall springs a leak, for example following tissue trauma, the high pressure becomes a problem, as it supports blood loss. In mammals, a sophisticated haemostatic surveillance pathway has evolved that almost instantaneously seals the damaged vessel wall, thereby preventing excessive blood loss and maintaining host integrity. Platelets and the fibrin-producing coagulation system, which mediate haemostasis, generate a clot that prevents blood loss and re-establishes vascular continuity. In this section, we briefly introduce the basic principles of mammalian haemostasis and its major players (FIG. 1). For a more comprehensive discussion of the haemostatic system the reader is referred to previous reviews[5,12].

***Platelet recruitment and activation.*** Platelets are small, disc-shaped anucleate cells (~1–3 μm in diameter) that are generated and released from megakaryocytes in the bone marrow[13,14]. Millions of platelets are produced every day that circulate in the blood and act as sentinels of vascular integrity. When vascular injury occurs, platelets are recruited to the damage site in a highly efficient, multistep process that involves the interaction of specific platelet cell-surface receptors with subendothelial matrix proteins (including collagens and von Willebrand factor) that are exposed or locally released in substantial amounts only following injury[5,12,15–18] (FIG. 1). After initial tethering steps, platelets become activated and firmly adhere to the vessel wall. Adherent platelets release potent platelet agonists (such as ADP) from their intracellular granules, which promotes paracrine platelet activation and receptor-mediated binding of additional platelets (a process termed platelet aggregation). The continued adhesion, activation and aggregation of platelets results in the rapid growth of the thrombus.

***The coagulation pathway.*** In parallel with the recruitment of platelets, the blood coagulation cascade is activated at the site of blood vessel injury[12]. Whereas platelet aggregation provides a provisional closure of the wound, coagulation ensures that it remains mechanically stable, sealing the defect using the glue-like substance fibrin, which is the major end product of the coagulation cascade. Fibrin formation requires the sequential activation of blood-based serine proteases and their cofactors. The formation of fibrin is initiated by a single cell-membrane protein termed tissue factor, which is expressed by vessel-wall cells that are not exposed to blood (such as adventitial fibroblasts) and by tissue cells[19] (FIG. 1). The endothelial barrier provides a spatial segregation between blood clotting factors and tissue factor in intact tissue. This ensures that tissue factor initiates the coagulation cascade only when the vessel wall is disrupted and tissue factor is exposed to blood, thereby enabling it to interact with a blood-based coagulation factor termed factor VIIa. The interaction of tissue factor with factor VIIa triggers the activation of coagulation factor X, resulting in the formation of activated factor X (factor Xa). Factor Xa in turn induces the production of thrombin, a central mediator of haemostasis. Thrombin promotes further factor X activation via a positive feedback loop and, most importantly, converts fibrinogen in the blood into fibrin. In order to prevent uncontrolled or disseminated coagulation, the coagulation process has to be tightly regulated by endogenous anticoagulant proteins, such as tissue factor pathway inhibitor (TFPI), vitamin K-dependent protein C and antithrombin[20].

Box 1 | **Evolutionary aspects linking haemostasis and inflammation**

The origins of blood coagulation in mammals can be traced back more than 400 million years. The process shares features with ancestral serine-protease cascades that are involved in the clotting of haemolymph in arthropods (that is, crustaceans and insects)[6–8]. In response to physical injury, the haemolymph of arthropods forms clots to provide rapid wound repair, resembling the role of haemostasis in mammals.

Wounds not only bring along the danger of loss of haemolymph, but also present an important port of entry for pathogens and hence put the host at great risk of infection. Thus, it is of crucial importance that the defence system of the host is activated as soon as wound sealing begins in order to prevent pathogen invasion and dissemination. Correspondingly, in arthropods, haemolymph clotting is also a component of their antimicrobial defence system[8]. The clotting process prevents the invasion of pathogens into organ cells by supporting the extracellular immobilization of the pathogens and may contribute to the production of antimicrobial peptides[8].

During evolution, the responses to injuries and to invading pathogens in arthropods and mammals have largely diverged. Mammalian organisms respond to physical injuries by activating haemostasis, whereas antimicrobial defences are mediated by distinct mechanisms of the innate and adaptive immune system. Despite this divergence, several observations suggest that blood coagulation and platelet activation could still be related to antimicrobial defence in mammals[6,7], conserving some aspects of their ancestral functions.

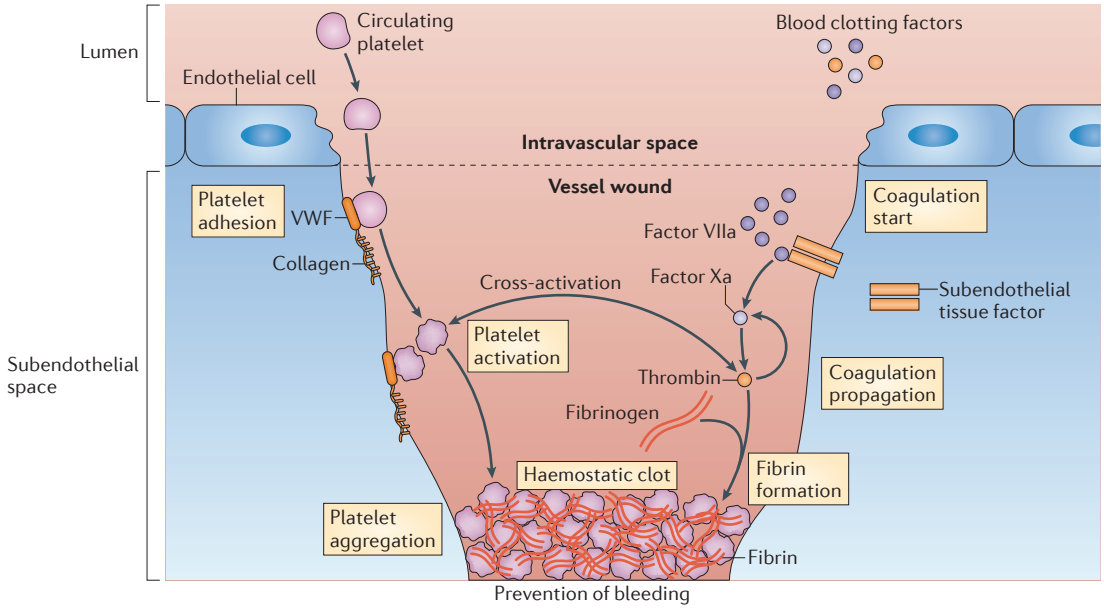


Figure 1 | **Mammalian haemostasis: a specialized process to prevent blood loss after injury.** Haemostasis involves two principle components: platelets, and the coagulation system with its major product, fibrin[12]. Both systems act in concert to generate a haemostatic clot that seals the wound. The efficient recruitment of platelets involves specific surface receptors, such as the platelet glycoprotein Ib (GPIb)–GPV–GPIX complex, GPVI and several integrins (not shown). These platelet receptors recognize distinct ligands that are normally concealed by the endothelial barrier and become exposed only after vessel damage. These ligands include von Willebrand factor (VWF) and collagens, as well as fibrinogen, vitronectin and fibronectin (not shown). Platelet recruitment does not induce haemostasis unless fibrin is also formed by the coagulation system. Coagulation requires the sequential activation of blood-based serine proteases and their cofactors (collectively known as blood clotting factors). The process is initiated by tissue factor, which is expressed by subendothelial cells and is therefore hidden in the intact vessel wall[19]. In response to injury, however, tissue factor is exposed to blood and can interact with blood-based factor VIIa to trigger the coagulation cascade, which culminates in the formation of fibrin.

***Cooperation of platelets and coagulation during haemostasis.*** The processes of platelet accumulation and blood coagulation promote each other during haemostatic plug formation at sites of vascular injury. Accordingly, platelets exert potent procoagulant functions via the calcium-dependent cell-surface exposure of phospholipids such as phosphatidylserine[21], which support coagulation by acting as cofactors for the proteolytic reactions that are triggered by coagulation factors. Coagulation in turn fosters platelet activation and accumulation, mainly through the protease thrombin, which promotes platelet activation via the cleavage and activation of proteinase-activated receptor 1 (PAR1) and PAR4 on human platelets[22]. Hence, throughout the formation of the haemostatic plug, platelets and the coagulation cascade communicate and mutually support each other.

## Thrombosis and its control by innate immune cells

As mentioned above, thrombosis is generally considered to be an aberration of haemostasis. Interestingly though, a distinct subset of cellular and molecular determinants that support thrombosis is largely irrelevant for blood vessel repair via haemostasis. In particular, the active participation of innate immune cells in clot formation is a specific feature of thrombosis. In this section, we highlight the current knowledge about the role of innate immune cells as effectors of thrombosis and describe the pathways involved (FIG. 2).

***Recruitment of innate immune cells to nascent thrombi.*** Both monocytes and neutrophils are rapidly recruited to the vessel wall and/or are incorporated into growing intravascular clots during thrombus development. The molecular and cellular cascades that initiate leukocyte accumulation during clot formation vary depending on the initial trigger of thrombosis and the phase of thrombus development. During arterial thrombosis, which is triggered by atherosclerotic plaque rupture leading to endothelial disruption, the recruitment of leukocytes occurs under high-shear conditions and is supported by platelets[12,23]. Platelets are first recruited to the exposed subendothelium in a process closely resembling physiological haemostasis (see FIG. 1) and, once activated, they express adhesion receptors (including P-selectin) and release chemokines that mediate the recruitment of innate immune cells[12,23,24].

By contrast, during the development of DVT, endothelial cells remain morphologically intact but become activated and adopt a pro-inflammatory phenotype that initiates the recruitment of innate immune cells[25–27]. This triggers the slow rolling, intraluminal crawling and adhesion of the innate immune cells in affected veins, which involves the same molecular mechanisms as leukocyte accumulation at other sites of sterile inflammation[28] (for example, the release of P-selectin from Weibel–Palade bodies[25]). Platelet accumulation during DVT formation is supported by the

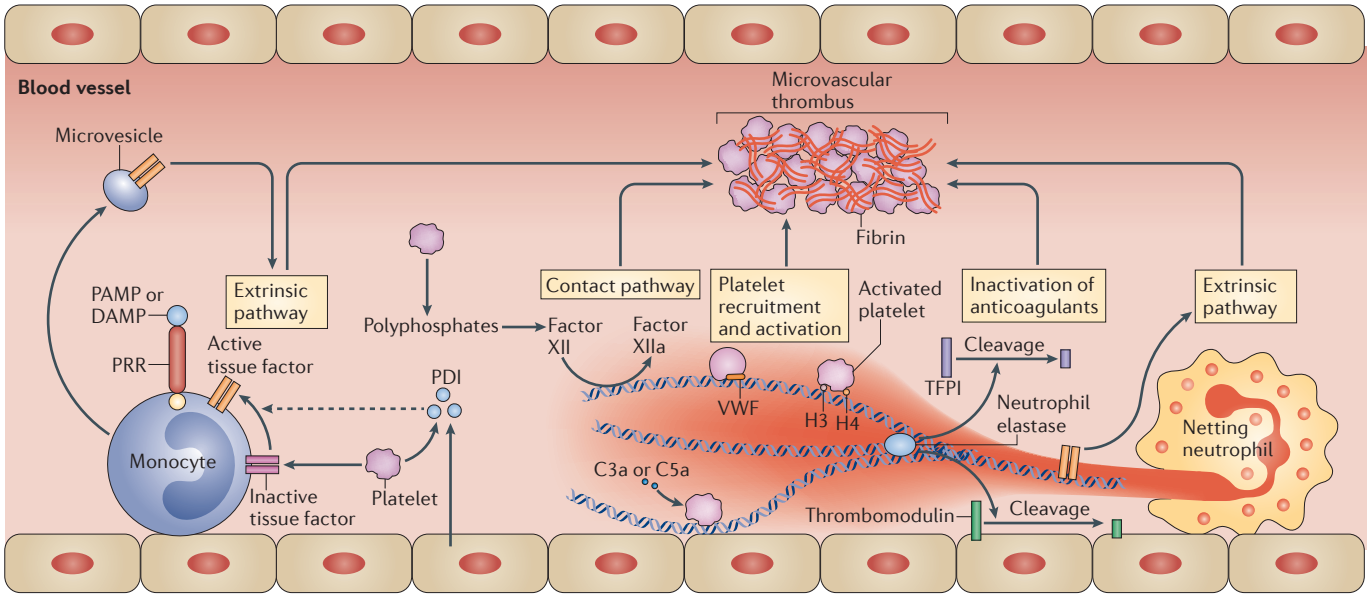


Figure 2 | **Basic principles of immunothrombosis.** Innate immune cells have evolved cell-specific prothrombotic pathways that operate in intact blood vessels to protect hosts from non-self and altered-self. In response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), monocytes and their microvesicles express and deliver activated intravascular tissue factor to sites of pathogen exposure, which initiates the extrinsic pathway of coagulation. Neutrophil extracellular traps (NETs) are lattices consisting of DNA and histones, and they support immunothrombosis through several pathways. One, NETs can directly activate factor XII (the contact pathway of coagulation), probably by virtue of their negatively charged surfaces. Two, NETs bind to von Willebrand factor (VWF) and support the recruitment of platelets. Three, histones H3 and H4, which are present in NETs, can trigger the activation of platelets. Four, NETs locally concentrate enzymes, such as neutrophil elastase and myeloperoxidase (not shown), which respectively cleave and oxidize anticoagulants, including tissue factor pathway inhibitor (TFPI) and thrombomodulin. Such inactivation of endogenous anticoagulants propagates coagulation. And finally, NETs can bind to tissue factor and promote the activation of the extrinsic pathway of coagulation. Platelets have an important supportive role in immunothrombosis. This role includes the activation of the factor XII-dependent contact pathway of coagulation by platelet-derived polyphosphates. In addition, platelet- and endothelial cell-derived protein disulphide isomerase (PDI) participates in fibrin generation, presumably via the activation of tissue factor (dashed arrow), although the exact molecular basis for the role of PDI in thrombus formation remains controversial. The complement system (specifically the activated complement components C3a and C5a) also supports immunothrombosis, for example by triggering platelet activation. PRR, pattern-recognition receptor.

inflammatory response and mainly involves receptor-dependent binding of platelets to neutrophils and monocytes, as demonstrated recently by real-time imaging in mice[25]. In addition, von Willebrand factor released by endothelial cells may promote direct platelet–endothelial cell interactions, a process that in turn fosters leukocyte recruitment during DVT[29].

***Platelets and the coagulation pathway activate innate immune cells.*** Within a growing clot, platelets and products of the coagulation pathway regulate the effector functions of the recruited innate immune cells. For example, PARs — which are expressed by many innate immune cells[22] — are activated by coagulation factors (such as thrombin and factor Xa) and induce pro-inflammatory outside-in signalling in dendritic cells[30]. During systemic infections, the concomitant activation of blood coagulation and inflammation has been suggested to represent a pathological 'vicious cycle'[20], with deleterious consequences for host tissues via the excessive activation of immune cells and intravascular thrombus formation (see below). Similarly, platelets release numerous mediators that support the recruitment and foster the microbicidal activities of leukocytes. Such mediators include CXC-chemokine ligand 1 (CXCL1), CXCL4, CXCL5, CXCL7, CC-chemokine ligand 3 (CCL3), CCL5, CCL7, the CD40 ligand CD154, and a ligand for triggering receptor expressed on myeloid cells 1 (TREM1)[24,31–33].

***Host molecules of specific relevance for thrombosis.*** Several molecules previously identified as necessary to support thrombosis are virtually dispensable for haemostasis. For example, some molecules implicated in platelet function have been suggested to be thrombosis specific. These include the platelet collagen receptor glycoprotein VI (GPVI)[15,34], which promotes platelet recruitment in arterial thrombosis, and growth arrest-specific protein 6 (GAS6), which belongs to the family of plasma vitamin K-dependent proteins and is a potent stimulator of platelet aggregation in both arterial and venous thrombosis[35,36].

In addition to the platelet-derived thrombosis-regulating proteins described above, a large number of thrombosis-related host molecules are produced or activated by monocytes and neutrophils (summarized in FIG. 2). Indeed, activated monocytes and the microparticles (or microvesicles) that they release express intravascular tissue factor (also known as blood cell tissue factor or blood-borne tissue factor) and can promote blood coagulation during thrombus development inside blood vessels[37–39]. Intravascular tissue factor might also be expressed by other types of immune cell, such as neutrophils[40,41] and eosinophils[42], as well as by platelets[38,43]. Intravascular tissue factor is mostly present in a functionally silent (encrypted) form that needs to be activated by distinct mechanisms, which probably include redox modification by the oxidoreductase protein disulphide isomerase (PDI). Even though the exact molecular basis for the role of PDI in thrombus formation remains controversial, several studies recently indicated that the secretion of PDI by activated platelets and endothelial cells or by damaged cells supports the activation of monocyte- and microvesicle-derived intravascular tissue factor (a process termed tissue factor decryption) and participates in fibrin generation across different models of arterial thrombosis[44–46] (BOX 2).

Interestingly, neutrophils have also evolved cell-specific mechanisms to potentiate thrombosis. When activated, they release neutrophil extracellular traps (NETs), which are comprised of a matrix of DNA and histones that is catapulted out of neutrophils. These NETs are decorated by several components of the antibacterial machinery of neutrophils, including myeloperoxidase, neutrophil elastase, pentraxin, lactoferrin, matrix metalloproteinase 9 (MMP9) and peptidoglycan recognition protein 1 (PGLYRP1; also known as PGRP-S)[47–50]. However, NETs not only serve antibacterial functions, but also induce a strong procoagulant response (FIG. 2). NETs can bind to and activate platelets in the setting of DVT[51]. In addition, the extracellular nucleosomes within NETs form a catalytic platform that stimulates the proteolytic activity of neutrophil elastase, which in turn promotes coagulation[52]. Correspondingly, neutrophil elastase deposited on NETs induces the degradation and inactivation of the anticoagulant molecule TFPI. The cleavage of TFPI by neutrophil elastase is supported by activated platelets that attach to the surface of neutrophils and facilitate NET formation[52]. Thus, neutrophil serine proteases release the brakes on the tissue factor-induced activation of coagulation (also termed the extrinsic pathway of coagulation). As a consequence, platelet–neutrophil conjugates can directly initiate coagulation via the enhancement of intravascular tissue factor activity[53].

In addition, neutrophils may degrade and modify other natural anticoagulants, including thrombomodulin, which is cleaved by neutrophil elastase and may also be rendered inactive by neutrophil oxidases[54,55], which are also found on NETs. Extracellular nucleosomes in NETs can also initiate fibrin formation more directly by inducing the activation of the contact pathway of coagulation, which involves the activation of the blood plasma-derived coagulation molecule factor XII to form factor XIIa[25]. Finally, the histone components of the extracellular nucleosomes in NETs can promote thrombosis through the activation of platelets via Toll-like receptor 2 (TLR2) and TLR4 (REFS 56–58).

Hence, innate immune cells, as well as platelets and the plasma compartment of blood, contain and release molecules and supramolecular complexes (such as nucleosomes and microvesicles) that are involved in thrombosis but are thought to be dispensable for the physiological process of haemostasis.

## Immunothrombosis promotes immune defence

Why do innate immune cells induce blood coagulation? It has been known for many years that coagulation has a role in the response to pathogen exposure[20,59]. Fibrin can exert direct antimicrobial effects and limit pathogen spreading[60]. Probably the first recognized link between mammalian blood coagulation and antimicrobial defence was the observation that fibrin deposition in extravascular locations, such as peritoneal cavities, contributes to the trapping of bacteria[60]. Mice lacking fibrinogen, the precursor of fibrin, succumb prematurely to pathogen infection[61]. In addition to confirming these findings, subsequent studies have indicated that fibrin and/or its precursor fibrinogen bind to and activate innate immune cells, such as neutrophils, at sites of infection[62–65]. This suggests that, in addition to its primary function in haemostasis, coagulation has a role in the host response to pathogen intruders.

The recent progress in identifying intrinsic prothrombotic functions of innate immune cells that operate inside blood vessels and are activated by contact with blood-borne microorganisms brings us to propose the concept of immunothrombosis. This physiological process is supported by the formation of microthrombi within microvessels. These microthrombi act as antimicrobial matrices that mediate host protection against pathogens[52] (FIG. 3). Immunothrombosis is triggered and maintained by the local accumulation of innate immune cells (especially monocytes and neutrophils), a process that is likely to involve the adoption of a pro-adhesive phenotype by microvascular endothelial cells that are exposed to pathogens[66].

However, it is still unknown why activated immune cells can induce thrombosis and why thrombosis-specific molecules have evolved in mammals, given that thrombosis can be a major threat to host organisms owing to its ability to suppress organ perfusion. The persistence of cellular and molecular mediators of thrombosis could represent an evolutionary 'dead end' that does not provide a benefit for host fitness. However, recent findings summarized below show that immunothrombosis in microvessels impedes the dissemination and tissue invasion of pathogens and enhances bacterial killing[52]. Hence, thrombosis-specific molecules have probably evolved and persisted to mediate immunothrombosis, thereby providing a benefit for host fitness by fighting pathogen invaders.

**Microparticles**
(Also known as microvesicles). Cell-derived membrane vesicles that originate from the plasma membrane via shedding. Microparticles have to be distinguished from exosomes, which are cell-derived membrane vesicles derived from multivesicular bodies, which are compartments of the endosomal system.

**Intravascular tissue factor**
Tissue factor that is expressed on cells in the blood and on the microparticles that they release.

**Neutrophil extracellular traps**
(NETs). Sets of extracellular fibres produced mostly by activated neutrophils to ensnare invading microorganisms. NETs enhance neutrophil-mediated killing of extracellular pathogens but cause minimal damage to host cells.

**Extracellular nucleosomes**
Extracellular lattices consisting of DNA and histones that can originate from neutrophils, in particular in the form of NETs.

**Extrinsic pathway of coagulation**
A process supporting blood coagulation that is traditionally assumed to be initiated outside the blood vessel lumen.

**Contact pathway of coagulation**
A process that supports blood coagulation initiated by blood-borne factor XII, which is activated following contact with pathogens, damaged cells and 'foreign surfaces' such as glass.

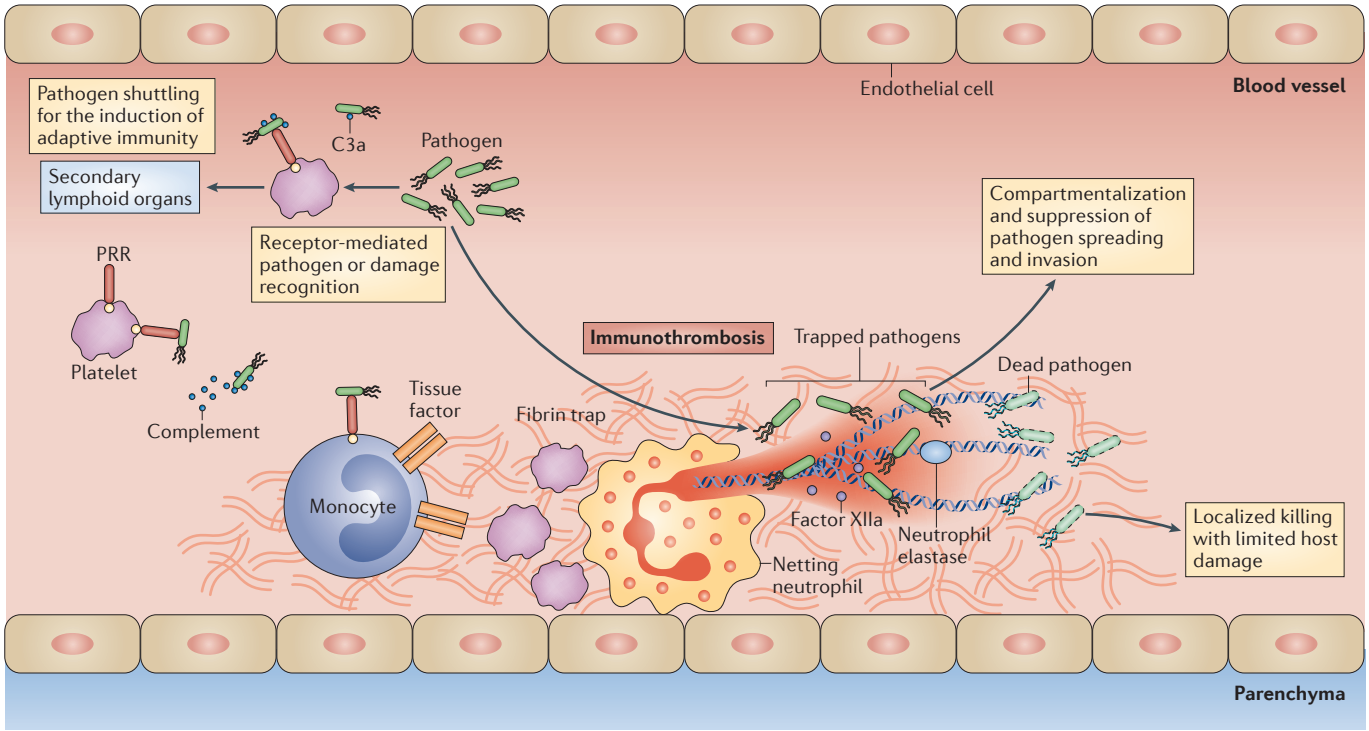


Figure 3 | **Retention of pathogens by immunothrombosis.** We propose the existence of a physiological form of thrombosis that supports innate immune defence against pathogens. Immunothrombosis and its central components provide all of the crucial defence strategies, including pathogen recognition, pathogen compartmentalization and trapping, prevention of pathogen spreading and invasion, and pathogen killing, as well as allowing for the establishment of an adaptive immune response and immune memory. During immunothrombosis, the combat against the pathogen is restricted to the intravascular compartment and thus triggers minimal host parenchymal damage. PRR, pattern-recognition receptor.

***Immunothrombosis involves coagulation in infection.*** The efficient and coordinated activation of intravascular coagulation in response to blood-borne pathogens and circulating products of tissue damage enables the biological process of immunothrombosis. During immunothrombosis, innate immune cells exploit their procoagulant repertoire, which includes the local delivery of active tissue factor, the degradation of endogenous anticoagulants and the provision of a procoagulant matrix consisting of extracellular nucleosomes (FIGS 2,3). All of these strategies are distinct from the pathways involved in haemostatic plug formation (FIG. 1).

Mice with severely impaired tissue factor expression have a reduced capacity to mount a coagulant response to pathogens, which is associated with an increased pathogen burden and decreased host survival[67]. This finding suggests that tissue factor that is expressed and delivered by monocytes, as well as by monocyte-derived microparticles, and presumably also by neutrophils, is the key initiator of coagulation associated with immunothrombosis[67]. The coagulation-triggering effect of tissue factor is converted into a longer-lasting activation of coagulation via the cleavage of its endogenous antagonist TFPI[52].

Of note, the activation of intravascular tissue factor is directly linked to the recognition of pathogens or damaged cells by leukocytes. For example, the detection of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), by receptors expressed by blood monocytes (including TLRs and CD14) leads to enhanced tissue factor gene transcription and protein expression[68]. Moreover, tissue factor is activated by molecules termed damage-associated molecular patterns (DAMPs) that are exposed or released from damaged host cells (including cell surface-exposed phosphatidylserine[69] and the injury signal PDI[44]). Thus, intravascular tissue factor activity is enhanced in response to pathogens and injured host cells through *de novo* synthesis of the tissue factor protein and through additional mechanisms that act on the protein after its insertion into the cell membrane (BOX 2). Notably, platelets also express PAMP receptors and promote coagulation in response to PAMPs[58]. Hence, the recognition of pathogens and damaged host cells is coupled to the induction of coagulation inside blood vessels during immunothrombosis. In addition, some elements of the coagulation system can directly recognize pathogens or damaged host cells. In particular, evidence suggests that factor XII may be activated by PAMPs[70] and by nucleic acids released from damaged cells[71].

Another major component of immunothrombosis is NETs, which act as catalytic surfaces that promote and compartmentalize the coagulation system. As discussed above, the procoagulant action of NETs involves the activation of factor XII[25] and the degradation of

anticoagulants such as TFPI and probably thrombomodulin[52,72]. Infusion of an antibody specific for histones H2A and H2B and DNA, which disrupts NETs, attenuates coagulation in the hepatic microcirculation of mice exposed to systemic *Escherichia coli* infection[52], whereas the administration of histones to mice promotes fibrin formation[56,57].

***Platelets recognize and respond to pathogens.*** In addition to the initiation and propagation of coagulation by innate immune cells, platelets (which are a core component of clots) facilitate immunothrombosis through multiple mechanisms. Indeed, platelets support the accumulation of innate immune cells and stimulate the upregulation of tissue factor expression, particularly on monocytes. In response to bacterial products, platelets directly bind to neutrophils and trigger the formation of NETs (a process termed NETosis)[52,73]. The exact mechanisms of platelet-driven NETosis remain to be established; however, β-defensins released by activated platelets have recently been reported to induce robust NET formation[74]. Although platelets facilitate and accelerate NETosis, NET formation can also occur in the absence of platelets[47]. The histone components of NETs, particularly histones H3 and H4, also feed back to platelets and promote platelet recruitment and activation[51,57]. Finally, platelets release DAMPs such as PDI, which can promote tissue factor decryption within thrombi[44], a process that is likely to foster immunothrombosis.

In addition, activated platelets have evolved antimicrobial strategies that can directly support the innate immune response to pathogens during immunothrombosis and beyond. For example, platelets bind to microorganisms and can become activated to assist in host defence during infections[24,33,52,73,75]. They are endowed with several pattern-recognition receptors, including all of the components of the LPS receptor complex, and can actively bind to circulating bacteria and present these microorganisms to neutrophils and other innate immune cells[76–80]. In addition, recent studies in mice have revealed that platelets cooperate with the complement systems to transport systemic bacteria to splenic CD8α$^+$ dendritic cells, a process that is essential for the induction of adaptive antibacterial immunity[81]. Notably, the complement system is a strong trigger of coagulation and platelet activation[82–84], and platelet products (such as chondroitin sulphate A) are potent initiators of the complement cascade[84]. Hence, the complement system and prothrombotic pathways mutually activate each other and could thereby also contribute to immunothrombosis. In addition, activated platelets can directly support the innate immune response to pathogens by secreting antimicrobial peptides[24,75]. For instance, the *Staphylococcus aureus* product α-toxin, which permeabilizes target cells, stimulates platelets to release β-defensins, which are antimicrobial peptides that curb microbial activity and impair bacterial growth. For a comprehensive discussion of the immune defence strategies provided by platelets the reader is referred to a recent review article[24].

Box 2 | **Mechanisms that activate tissue factor**

Tissue factor is a cell-membrane protein that serves as the primary initiator of blood coagulation. Tissue factor is often present in a functionally inactive form, especially when expressed by blood cells and on microparticles. Several mechanisms have been proposed to mediate the activation of tissue factor. These include: one, changes in the membrane environment surrounding tissue factor, in particular the translocation of phosphatidylserine to the outer leaflet of the cell membrane[69]; two, disulphide bond formation between a cysteine pair present in the extracellular domain of tissue factor, which could be mediated by protein disulphide isomerase (PDI)[44]; and, three, inactivation of tissue factor pathway inhibitor (TFPI), for example through proteolysis[52].

Platelets therefore serve an important supportive function in immunothrombosis, in that they recognize pathogens and enhance the prothrombotic functions of innate immune cells.

***Immunothrombosis promotes defence against pathogens.*** We suggest that the process of immunothrombosis is a major element of an intravascular innate immune system and serves at least four different physiological functions. One, it helps to capture and ensnare pathogens circulating in the blood and thereby limits pathogen dissemination by retaining the microorganisms within the fibrin network. Two, it prevents tissue invasion by pathogens through the formation of microthrombi in microvessels[52]. Three, the intravascular thrombi generate a distinct compartment that concentrates antimicrobial strategies and their pathogen targets and thus favours pathogen killing. This killing involves antimicrobial strategies, which are supplied by innate immune cells, and antimicrobial peptides that are generated during the activation of blood coagulation[85] and/or released by activated platelets[33] at sites of pathogen immobilization. Four, the microvascular accumulation and deposition of fibrinogen or fibrin promotes the recruitment of additional immune cells to the site of tissue infection and/or damage, further supporting the recognition of pathogens and coordinating the immune response[62] (FIG. 3). Indeed, fibrin formation in response to several blood-associated pathogens, including *E. coli*[52] and *Yersinia enterocolitica*[67], has been shown to have a role in host protection.

NETs seem to be a crucial feature of immunothrombosis, and many of the antibacterial properties of NETs depend on the procoagulant functions of this DNA scaffold. Correspondingly, NET-driven bacterial capture is ablated by anticoagulants or by interference with the procoagulant machinery of NETs[52]. The importance of NET formation in human host defence is supported by recent observations in patients with chronic granulomatous disease. These patients lack NADPH oxidase and as a consequence are incapable of forming NETs, and this is associated with their increased susceptibility to aspergillosis[86].

Sepsis
A systemic response to severe infection or tissue damage that leads to a hyperactive and unbalanced network of pro-inflammatory mediators. Vascular permeability, cardiac function and metabolic balance are affected, resulting in tissue necrosis, multi-organ failure and death.

It seems noteworthy that the antimicrobial mechanisms supported by intravascular fibrin formation and blood cell deposition do not *per se* seriously impair organ function. This is evidenced by the absence of any severe damage to organ cells (for example, hepatocytes) caused by immunothrombosis during bacterial infection[52]. This is probably explained by the fact that immunothrombosis obstructs only a limited percentage of microvessels, and thus nutritive blood perfusion is maintained.

Immunothrombosis, and its procoagulant machinery, thus supports the basic mechanisms required for innate immune systems[87]: pathogen recognition; the suppression of pathogen spreading; and the restriction of pathogen survival at the expense of limited damage to host cells (FIG. 3). This suggests that immunothrombosis constitutes a newly identified pathway of innate immunity.

## Pathogens subvert immunothrombosis

Evolution has endowed several pathogens with sophisticated strategies that enable them to evade coagulation-induced antimicrobial mechanisms and assist them to exploit immunothrombosis (TABLE 1). For example, group A, C and G streptococci express streptokinase, which activates host plasminogen to form plasmin, the major serine protease that dissolves fibrin[88]. As a consequence, group A streptococci use streptokinase to invade and spread into host tissues[89], possibly through their ability to prevent immunothrombosis. In addition, several pathogens have adopted an evolutionary strategy to escape prothrombotic NETs. Prominent examples are *Streptococcus pyogenes* and *Streptococcus pneumoniae*, which cause invasive infections, including necrotizing fasciitis and pneumonia[90,91]. Both pathogens express a DNase that degrades NETs and inhibits NET-mediated killing.

Some microorganisms exploit fibrin formation and immunothrombosis in order to escape detection by the immune system. For example, staphylocoagulase and von Willebrand factor-binding protein are virulence proteins of *S. aureus* that can stimulate fibrin generation through the activation of prothrombin. *S. aureus* uses fibrin to build a multilayered barrier against invading immune cells, which forms part of the abscess pseudocapsule that is typically induced by this pathogen[92,93]. In addition to activating blood coagulation, *S. aureus* promotes platelet aggregation by releasing clumping factor A (CLFA) and CLFB[33], which protect this pathogen from host clearance.

## The risks of aberrant immunothrombosis

Although immunothrombosis is suggested to support pathogen recognition and host defence, aberrant or uncontrolled activation of immunothrombosis may be detrimental to the host. In this section, we summarize current evidence indicating that dysregulated immunothrombosis constitutes a major biological process underlying different forms of pathological thrombosis, including stroke, myocardial infarction, DIC during sepsis and DVT (FIG. 4).

***Uncontrolled activation of immunothrombosis during sepsis.*** Blood-borne infections can result in sepsis and thereby cause life-threatening organ failure. Sepsis is associated with DIC, a condition that promotes the activation of blood coagulation and microvessel thrombosis in various organs[59]. Similarly to immunothrombosis, DIC occurs in microvessels, is induced in response to systemic infections, and is initiated and sustained by thrombosis-specific molecules, such as intravascular tissue factor[94] and NET-associated nucleosomes[95]. In addition to infections, the massive release of injured cells and their fragments into the blood circulation during trauma can cause DIC[96]. Hence, DIC may be triggered when immunothrombosis becomes overwhelmed and is no longer able to restrict the spread of pathogens or damaged cells. This failure of immunothrombosis results in the unrestricted formation of microvessel thrombi and the excessive activation of inflammation, which is aggravated by the ability of both processes to potentiate each other[20]. Immunothrombosis could thus represent an initial physiological stage in the development of DIC (FIG. 4).

Table 1 | **Examples of the subversion and exploitation of immunothrombosis by pathogens**

| Pathogen | Pathogen-derived mediator | Mechanism of action | Refs |
|---|---|---|---|
| ***Subversion of coagulation*** | | | |
| β-haemolytic group A, C and G streptococci | Streptokinase | Fibrinolysis via plasmin formation, resulting in fibrin degradation | 88,89 |
| β-haemolytic *Streptococcus pyogenes* | DNase | Degradation of prothrombotic NETs | 90 |
| α-haemolytic *Streptococcus pneumoniae* | DNase | Degradation of prothrombotic NETs | 91 |
| ***Exploitation of coagulation*** | | | |
| *Staphylococcus aureus* | Staphylocoagulase and VWF-binding protein | Activation of prothrombin, leading to fibrin generation and preventing pathogen clearance | 92,93 |
| *Staphylococcus aureus* | CLFA and CLFB | Promotion of platelet aggregation, hampering pathogen clearance by host immune cells | 33 |

CLFA, clumping factor A; CLFB, clumping factor B; NET, neutrophil extracellular trap; VWF, von Willebrand factor.

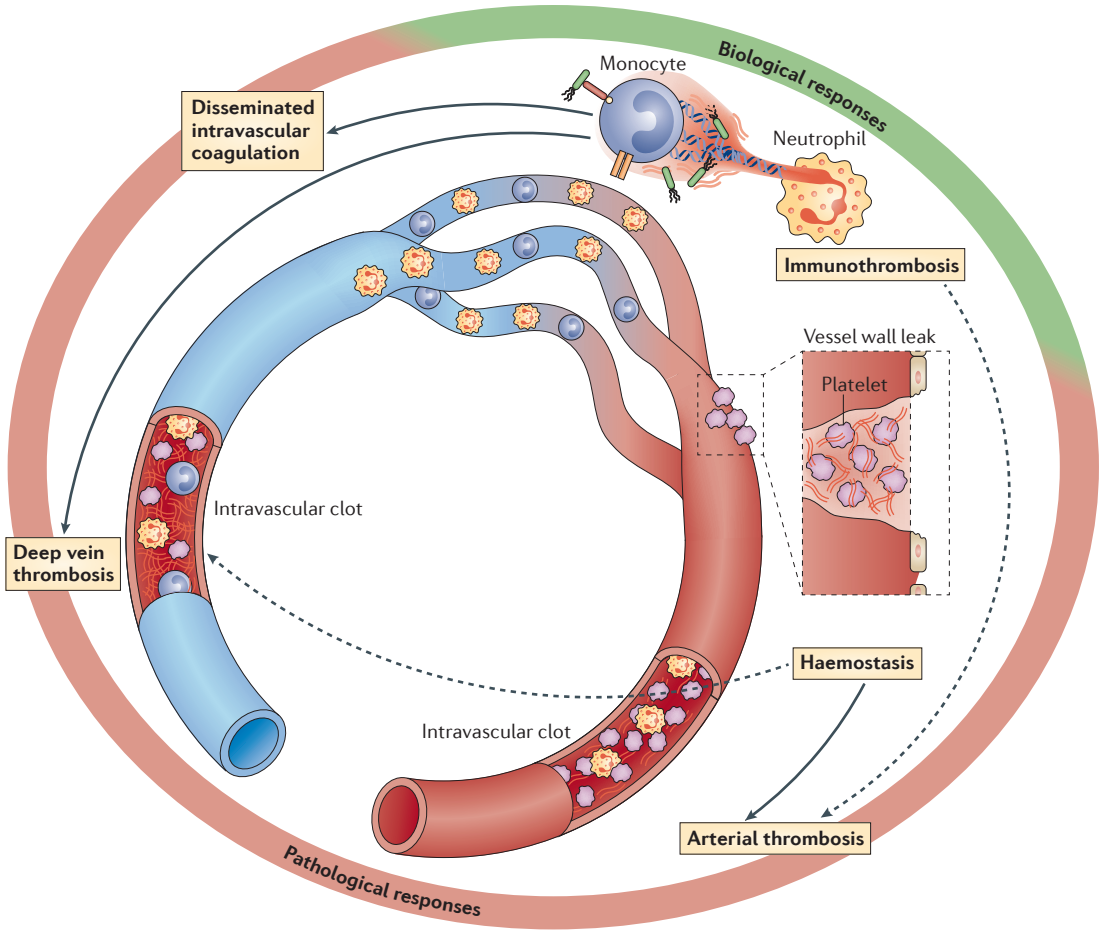


Figure 4 | **The thrombotic continuum — uncontrolled haemostasis and immunothrombosis trigger disease.** Both haemostasis and immunothrombosis are biological processes that serve to maintain host integrity (indicated by the green colour). Whereas haemostasis is essential for preventing blood loss whenever the vessel leaks, immunothrombosis — the local formation of thrombi (clots) in microvessels, supported by fibrin generation and the recruitment of immune cells and platelets — represents a mechanism of intravascular antimicrobial defence. Dysregulation of haemostasis and immunothrombosis constitutes the major biological mechanism underlying different forms of pathological thrombosis in disease states, including disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT), myocardial infarction and stroke (indicated by the red colour). DIC may result when immunothrombosis is no longer able to restrict the spreading of pathogens or of damaged cells. Aberrant activation of haemostasis, and also of processes resembling those involved in immunothrombosis (dashed arrow), trigger arterial thrombosis (that is, clot formation inside large arteries). Likewise, uncontrolled activation of immunothrombosis inside large veins, assisted by aberrant activation of coagulation (dashed arrow), is likely to be a major trigger of DVT.

***Contribution of aberrant immunothrombosis to atherothrombosis.*** The primary trigger of arterial thrombosis is the rupture of an atherosclerotic plaque, which consists to a large extent of an accumulation of inflammatory cells and lipid deposits. Plaque rupture triggers platelet adhesion, activation and aggregation[12,36]. The platelet receptors and subendothelial ligands that promote platelet accumulation during arterial thrombosis are similar to those involved in haemostatic clot formation. These receptors include the GPIb–GPV–GPIX complex (which binds to von Willebrand factor), GPVI and integrin α2β1 (which bind to collagens), integrin α5β1 (which binds to fibronectin), integrin α6β1 (which binds to laminin) and integrin αIIbβ3 (which binds to von Willebrand factor, fibrin and fibrinogen)[36]. Although arterial thrombosis is initiated by platelets, the activation of coagulation and subsequent fibrin formation have a crucial role during thrombus stabilization and thrombus growth. Although activated platelets can directly support blood coagulation (for example, through the release of inorganic polyphosphates[97], which are polymers comprising 60–100 phosphate residues), robust fibrin formation throughout the growing thrombus requires the participation of innate immune cells. Correspondingly, studies in baboons have shown that the ablation of neutrophil and monocyte recruitment is associated with the virtual abrogation of fibrin formation and leads to thrombus destabilization[23].

Recent evidence suggests that the mechanisms used by immune cells to trigger coagulation in nascent arterial thrombi partially mimic those involved in immunothrombosis (FIG. 4). In particular, this pertains to the participation of intravascular tissue factor. The tissue

factor in plaques — which is generally regarded as the prime trigger of coagulation after plaque rupture — is mostly associated with innate immune cells that originated from the blood[19]. In addition, blood cells deliver tissue factor locally to the growing arterial thrombus[98,99].

Finally, there are other trigger mechanisms of specific relevance that support both large-vessel thrombosis in arteries and immunothrombosis. These mechanisms include the release of nucleosomes by neutrophils[52], the local degradation of endogenous anticoagulants[52] and the production of factor XIIa[100,101]. The ablation or inhibition of effectors of immunothrombosis (such as extracellular nucleosomes or factor XIIa) affords protection against pathological thrombosis in large arteries. Current strategies to treat or prevent arterial thrombosis — which consist of a combination of antiplatelet and anticoagulant agents — target major haemostatic pathways and thus share the inherent weakness of having deleterious effects on bleeding. Although evidence from humans is still lacking, experiments in mice have shown that the protective effects of inhibiting factor XII are not associated with defective haemostatic function. This suggests that targeting triggers of immunothrombosis could help to maximize the efficacy of therapies that prevent arterial thrombosis without increasing the incidence of bleeding complications.

***Venous thrombosis and the role of immunothrombosis.*** The molecular and cellular determinants that support the development of DVT are closely related to those that induce immunothrombosis. Indeed, the pathogenesis of DVT illustrates quite precisely how uncontrolled activation of immunothrombosis can become a liability with deleterious effects for host integrity (FIG. 4). With an annual incidence of 2–3 cases per 1,000 individuals[2], DVT and its major complication, pulmonary embolism, constitute the third leading cause of cardiovascular death globally. Thrombi that form in deep veins are rich in fibrin and contain layers of platelets, red blood cells and leukocytes. The cascade of events leading to DVT has remained incompletely understood, largely owing to the lack of a relevant animal model. However, recent data obtained in mice suggest that aberrant activation of immunothrombosis is a key event in the initiation and propagation of DVT. Indeed, real-time observation of nascent venous thrombi revealed that DVT starts as sterile inflammation with a massive recruitment of neutrophils (see Supplementary information S1 (movie)) and monocytes[25]. In addition, this study[25] showed that the delivery of intravascular tissue factor by myeloid cells — a major mechanism of immunothrombosis — is indispensable for DVT formation. Indeed, mice lacking tissue factor on myeloid cells were protected from DVT formation. NETs also have a crucial involvement in DVT formation. However, the mechanisms that contribute to NET-driven venous thrombosis differ from those supporting arterial thrombosis and include the activation of the contact pathway of coagulation, the binding of von Willebrand factor to NETs and the recruitment of platelets[25,51]. Correspondingly, the infusion of histones, which are key components of NETs, has been reported to promote DVT formation[102]. Although platelets are less frequent in venous thrombi than in arterial thrombi, they have an important role during DVT formation, as they further support aberrant immunothrombosis by interacting with neutrophils to promote the generation of NETs[25]. However, it remains to be established whether aberrant immunothrombosis contributes to all forms of DVT or is restricted to certain subgroups of DVT[27]. Finally, whether the pathways involved in immunothrombosis are also responsible for thrombotic complications of other disease entities — such as systemic lupus erythematosus, a disorder associated with an increased occurrence of NETs[103,104] — will have to be evaluated in future studies.

## Conclusion and perspective

The presence of microorganisms inside blood vessels is a major threat to the host organism, as circulating pathogens can concomitantly infect several organs that are crucial for survival. Immunothrombosis — the local formation of thrombi in microvessels supported by fibrin generation and the recruitment of immune cells and platelets — represents a mechanism of intravascular antimicrobial defence. This process is specialized to suppress the dissemination and tissue invasion of pathogens and the circulation of damaged host cells. The involvement of immunothrombosis-associated cellular mediators (for example, neutrophils) and molecular mediators (for example, intravascular tissue factor) in pathological thrombosis suggests that the dysregulation of immunothrombosis forms a decisive biological basis for thrombotic disorders, including venous thromboembolism, atherothrombosis and aberrant coagulation activation associated with sepsis. Hence, large-vessel thrombosis, the most frequent lethal disease worldwide, is to a major extent a direct consequence of a dysregulated intravascular innate immune process. The identification of the specific biological signature underlying pathological thrombosis expands our understanding of its pathogenesis and consequently should facilitate the development of new diagnostic and therapeutic tools. Future work will need to further establish the importance of immunothrombosis for host protection and to further characterize the host molecules involved in this process.


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### Competing interests statement
The authors declare no competing financial interests.

### FURTHER INFORMATION
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