Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

PORTLAND PRESS

Review Article

Dysregulated haemostasis in thrombo-inflammatory disease

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Inflammatory disease is often associated with an increased incidence of venous thromboembolism in affected patients, although in most instances, the mechanistic basis for this increased thrombogenicity remains poorly understood. Acute infection, as exemplified by sepsis, malaria and most recently, COVID-19, drives 'immunothrombosis', where the immune defence response to capture and neutralise invading pathogens causes concurrent activation of deleterious prothrombotic cellular and biological responses. Moreover, dysregulated innate and adaptive immune responses in patients with chronic inflammatory conditions, such as inflammatory bowel disease, allergies, and neurodegenerative disorders, are now recognised to occur in parallel with activation of coagulation. In this review, we describe the detailed cellular and biochemical mechanisms that cause inflammation-driven haemostatic dysregulation, including aberrant contact pathway activation, increased tissue factor activity and release, innate immune cell activation and programmed cell death, and T cell-mediated changes in thrombus resolution. In addition, we consider how lifestyle changes increasingly associated with modern life, such as circadian rhythm disruption, chronic stress and old age, are increasingly implicated in unbalancing haemostasis. Finally, we describe the emergence of potential therapies with broad-ranging immunothrombotic functions, and how drug development in this area is challenged by our nascent understanding of the key molecular and cellular parameters that control the shared nodes of proinflammatory and procoagulant pathways. Despite the increasing recognition and understanding of the prothrombotic nature of inflammatory disease, significant challenges remain in effectively managing affected patients, and new therapeutic approaches to curtail the key pathogenic steps in immune response-driven thrombosis are urgently required.

Haemostatic response to vessel injury

Blood coagulation is activated in response to blood vessel injury (Figure 1). Traumatic disruption of the endothelial cell layer causes the recruitment of platelets to the site of vessel injury, either via platelet glycoprotein 1b receptor interaction with plasma von Willebrand factor (VWF) bound to sub-endothelial tissue, or via direct platelet collagen receptor binding to exposed collagen. Adherent platelets become activated, prompting extensive morphological changes and secretion of granular contents to encourage further platelet recruitment to the growing clot and provide a cellular surface to amplify procoagulant enzymatic reactions. Activated platelets also secrete VWF and express P-selectin, an important cell adhesion receptor, which binds to P-selectin glycoprotein ligand 1 (PSGL-1) expressed by monocytes and neutrophils. In parallel, vessel injury causes the exposure of tissue factor (TF), expressed extensively within the sub-endothelial tissue, to blood (for detailed review, see [1]). TF bound to activated FVII (FVIIa) triggers the formation of the extrinsic tenase complex (TF, FVIIa, and factor X, FX). Activated FX (FXa), bound to its cofactor activated factor V (FVa), promotes the conversion of zymogen prothrombin to thrombin. Thrombin, in turn, activates several coagulation proteases and cofactors, including FV, FVIII, and FXI, to

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Received: 03 September 2022
Revised: 17 November 2022
Accepted: 25 November 2022

Version of Record published: 16 December 2022

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

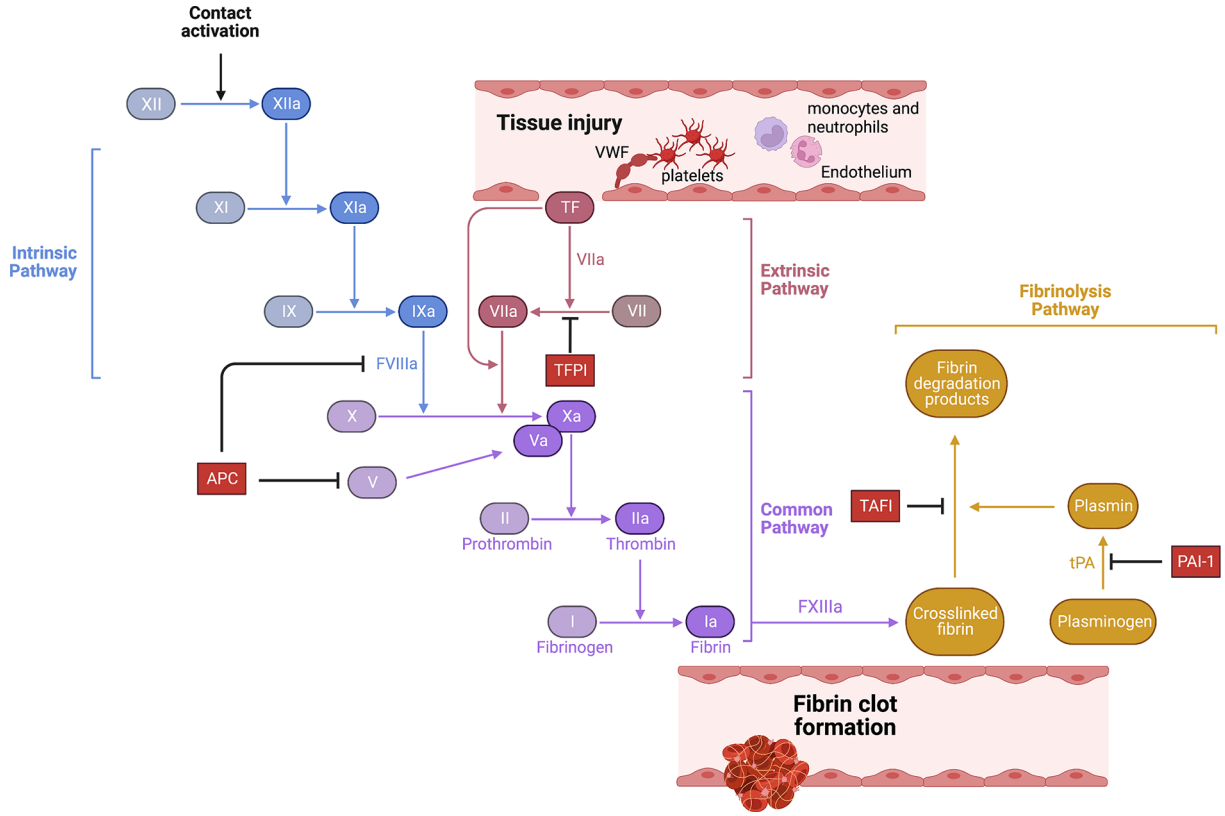

Figure 1. Mechanisms of blood clot formation

Blood coagulation is triggered by vessel injury and TF exposure. This triggers extrinsic pathway activation, leading to thrombin generation via the shared pathway and fibrin generation. Alternatively, contact pathway activation via FXII activation by polyP also leads to intrinsic pathway activation, thrombin generation and fibrin deposition in the absence of vessel damage, leading to pathological thrombus formation. Clot growth and size is regulated at multiple stages by endogenous anticoagulants, including tissue pathway inhibitor, the PC pathway and antithrombin. Once the vessel repair is underway, proteolytic clot lysis is orchestrated by plasmin, which breaks down cross-linked fibrin into fibrin degradation products.

amplify the procoagulant response and overwhelm counteractive anticoagulant mechanisms such as tissue factor pathway inhibitor (TFPI). FXIa activation of FIX prompts intrinsic tenase complex formation (FIXa, FVIIIa, and FX) that promotes sufficient FXa generation to drive prothrombinase activity, allowing generated thrombin to convert soluble fibrinogen in the blood into an insoluble fibrin mesh clot. Thrombin also further activates platelets and endothelial cells via protease-activated receptor (PAR1) signalling [2]. Endogenous anticoagulant mechanisms, such as antithrombin and the protein C (PC) pathway, regulate clot size and spread. In addition, fibrinolytic factors, such as tissue-type plasminogen activator (tPA) and plasmin, coordinate clot resolution by enzymatic fibrin degradation.

Pathological venous clot formation, typically in the absence of traumatic injury, requires factor FXII (XII) activation rather than solely TF-dependent fibrin generation (for detailed review, see [3]). FXII can be activated by negatively charged synthetic materials such as glass, silica or nanoparticles [4]. Recently, however, potential endogenous FXII activators have been identified, such as misfolded protein aggregates, free collagen, glycosaminoglycans and circulating nucleic acids [5]. Of particular importance is inorganic polyphosphate (polyP) [6], which is released from activated platelets to rapidly activate FXII and drive thrombus development [7].

What is ‘immunothrombosis’?

Clot development likely evolved in part as an ancient anti-bacterial response to enable localisation and neutralisation of various bloodborne pathogens. Aberrant clot formation, however, that arises from infection or excessive inflammatory response during disease is now commonly termed ‘immunothrombosis’ (Figure 2). Whereas this primitive response represents an effective strategy to slow pathogen spread during infections, this may come at the cost of immune-driven pathological thrombus formation. Currently, therapeutic approaches to control immunothrombotic

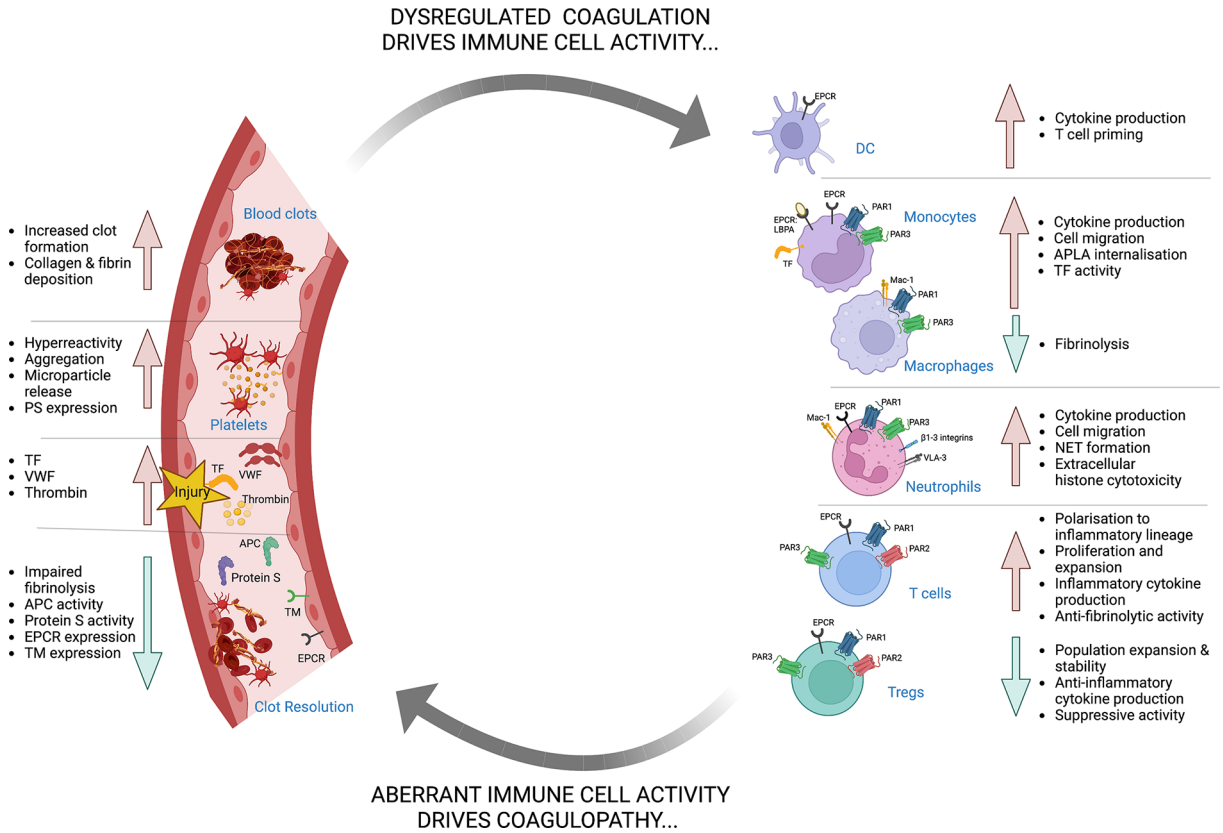

Figure 2. Molecular and cellular mechanisms of immunothrombosis

Both acute and chronic inflammatory diseases, including autoimmune diseases, allergies, and infections, promote dysregulation of haemostasis, leading to immunothrombosis. Inflammatory mediators such as enhanced pathogenic immune cell populations, increased proinflammatory cytokines and chemokines levels, and diminished tolerogenic Treg cell responses coincide with and promote aberrant coagulation. This enhanced procoagulant state feeds back to amplify inflammation, inducing further expansion of proinflammatory immune cells, enhanced TF expression in myeloid cells, NET production and inhibition of tolerogenic Treg responses that impair thrombolysis and clot resolution.

disease are limited by our nascent understanding of how proinflammatory and procoagulant pathways intersect. In this review, we describe the convergent mechanisms that determine how inflammatory events precipitate an increased risk of venous thromboembolism (VTE).

Activation of the immune response

Innate immune cells, such as monocytes, macrophages, granulocytes and dendritic cells (DCs), classically perform sentinel roles in immunosurveillance and antigen presentation to cells of the adaptive immune system, thereby inducing a robust immune response and generating immune memory [8]. These innate cells recognise intra- and extra-cellular pathogens via pathogen recognition receptors (PRRs). PRRs are sub-classified as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (Nod) like receptors (NLRs), C-type lectin receptors (CLRs), and RIG I-like receptors (RLRs). Macrophages and DCs recognise extracellular pathogens by sensing components of their cell wall such as LPS, bacterial lipopeptides, lipoteichoic acids, glycolipids, flagellin, and β-glucans via TLR1, TLR2, TLR4, TLR5, TLR6, and CLRs, such as dectin-1 and dectin-2, which are expressed on their plasma membrane [8]. TLR3, TLR7, TLR8, and TLR9, are expressed in the cell endosomes and sense intrinsic pathogens, such as viral nucleic acids [8]. RLRs, NLRs, and cytosolic DNA sensors detect viral RNA and DNA, and invading bacteria in the cytosol [8–12]. In the bloodstream, monocytes and neutrophils recognise invading bacterial, fungal and protozoan pathogens via TLRs, NLRs, and CLRs and facilitate phagocytosis, degranulation, and killing of these microorganisms [8,13]. Following pathogen recognition, innate immune cells also secrete inflammatory cytokines and chemokines

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

into the vicinity, recruiting both other innate and adaptive cells to the site to amplify the inflammatory response. Furthermore, antigen-loaded DCs migrate to the draining lymph nodes to prime an adaptive T-cell response. The type of T-cell response induced depends on several factors including, the location of the site of injury or infection, the class of antigen being presented and the presence of other instructive cytokines and chemokines in the milieu [8]. Typically, bacteria, protozoa and fungi elicit a type 1 immune response via TLRs, RLRs, and CLRs, involving CD4+ Th1 and Th17 cells, and CD8+ cytotoxic lymphocytes (CTLs) [14–19], whereas helminths, allergens, and venoms elicit a type 2 immune response, involving CD4+ Th2 cells [20–22]. CTL induction of type 1 immunity is also required to clear viral infection via RLRs, TLR3, and CLRs [23–25]. Recent studies have highlighted the role of both adaptive and innate immune cell responses in dysregulated haemostasis.

Immune cell contribution to pathological thrombus formation

Platelets represent the classical bloodborne haemostatic cell, and their prothrombotic activity is critical for normal haemostasis. In addition to platelets, recent data have highlighted the roles played by various innate immune cells in thrombus development via distinct and often cell-specific mechanisms. Post-mortem studies and preclinical deep vein thrombosis (DVT) mouse models suggest an essential contribution of myeloid cells to VTE. Neutrophils have a crucial role in response to infection and subsequent resolution of inflammation but have recently also been identified to be critical for DVT formation. Neutrophils release neutrophil extracellular traps (NETs), which represent a last-ditch 'damage-limitation' approach to kill microbes during infection [26] but can also induce toxic collateral damage within the vasculature. As such, NET formation is implicated in the pathogenesis of numerous autoimmune and inflammatory diseases [26–29]. Furthermore, NETs have been demonstrated to contribute to aberrant thrombus development via multiple mechanisms [30,31]. DNA strands within NETs have been reported to capture and contribute to FXII activation [32]. Notably, NET formation can be regulated by different plasma DNAses [33]. Moreover, neutrophil enzymes, including elastase and cathepsin G, can cleave and inactivate plasma TFPI, removing a vital anticoagulant regulator from the proximity of the developing thrombus [32]. Extracellular histones within NETs promote activation of platelets [34] and interact with NET-bound VWF [35]. In addition, extracellular histones inhibit the PC anticoagulant pathway by attenuating activated protein C (APC) generation by the thrombin–thrombomodulin complex on endothelial cells [36]. Therapeutic strategies to inhibit or degrade NETs have also been reported. For example, NET inhibitors that target protein arginine deiminases (PAD4) critical for NET formation exert beneficial activity in preclinical models of arthritis, shifting the T-cell balance from a proinflammatory Th1/Th17 response to a pro-resolution Th2 response [37].

Monocytes and tissue-resident macrophages also contribute to VTE development and monocyte count have recently been reported to correlate with thrombus size and growth in murine thrombosis models [38]. Myeloid cells typically express TF on their surface, and TF expression and activity are up-regulated by exposure to either pathogen-associated molecular patterns or proinflammatory cytokines [39]. In a mouse model of stasis-induced DVT, monocyte TF was critical for initiating DVT formation [40], highlighting the complex and multi-faceted role played by innate immune cells in pathological clot formation.

Coagulopathy during acute infection

Sepsis develops due to a systemic dysregulated immune response to acute infection and accounts for ∼20% of global deaths [41]. The case fatality rate of sepsis has steadily decreased in the past 20 years; however, its incidence is the same or increasing [42–44]. Sepsis is characterised by excessive cytokine production in response to infection, leading to tissue and organ dysfunction [45]. A prominent feature of sepsis is haemostatic dysregulation, thrombocytopenia, and disseminated intravascular coagulation (DIC) [46]. DIC is associated with a worse outcome and higher mortality rate compared with sepsis patients without DIC [47–49].

Aberrant up-regulation of TF expression on circulating innate immune cells and the endothelium upon exposure to proinflammatory stimuli has long been considered an important trigger for DIC [50–52]. Moreover, recent studies have provided a mechanistic basis for cell surface TF release into the blood during bacterial infection, highlighting the crucial role of non-canonical inflammasome formation in modulating TF-rich microparticle release from activated myeloid cells. This mechanism may also be partially conserved in other cell types, such as tumour cells, which also actively release TF-containing microparticles and are associated with increased thrombgenicity [53]. Activation of the canonical or non-canonical inflammasome in macrophages following exposure to *Escherichia coli* EprJ type-III-secretion system rod proteins or LPS, respectively, results in the release of TF-rich microparticles [54]. In this process, pathogen exposure triggers gasdermin D (GSDMD) cleavage by activated caspase-1 and -11 to drive pyroptosis and interleukin-1β (IL-1β) release [54]. It has also been reported that a GSDMD-dependent increase in TF activity

following non-canonical inflammasome activation occurs independently of cell death via increased phosphatidylserine (PS) exposure, aiding in the assembly of the extrinsic tenase complex [55]. By labelling PS on mouse splenic and peripheral blood leukocytes with lactadherin, the study showed increased PS on mouse leukocytes following transfection of LPS and a reduction in exposed PS in *Gsdmd*$^{-/-}$ leukocytes [55]. Furthermore, myeloid cell inflammasome formation has been shown to contribute to VTE development in a mouse inferior vena cava (IVC) stenosis model [56]. Mice with caspase-1 or GSDMD deficiency exhibited smaller and fewer thrombi formation following IVC stenosis compared with wild-type mice, underscoring the potential importance of canonical and non-canonical inflammasomes in immunothrombosis.

Similarly, pathogen and cytokine activation of the endothelium also contributes to a prothrombotic milieu during sepsis. Various cytokines promote the release of long, multimeric chains of ultra-large von Willebrand factor (UL-VWF) on to the endothelial cell surface. UL-VWF binds circulating platelets via platelet glycoprotein Ibα [57] and is typically broken down by the plasma metalloproteinase ADAMTS13 into smaller multimers, reducing VWF thrombogenicity [58]. UL-VWF can act as an adhesive surface to monocytes and polymorphonuclear leukocytes by binding PSGL-1 and β2-integrin [59]. VWF strings decorated with platelets and other immune cells have been suggested to contribute to microthrombi development in DIC. Notably, sepsis patients have lower levels of circulating ADAMTS13 and ADAMTS13 activity [60,61], contributing to sustained VWF strings in the intravascular space. In support of an essential role for ADAMTS13 in regulating VWF thrombogenicity during infection, low ADAMTS13 activity in patients with *Staphylococcus aureus* infections is associated with increased disease severity [62]. NETs formed during sepsis contribute to widespread organ damage [63] and DIC development [64,65] and their presence is reported to be especially deleterious in infant sepsis [66].

Many endogenous plasma anticoagulant proteins and cell receptors are depleted during sepsis, leading to unregulated thrombin activity and fibrin generation. For example, low levels of circulating PC, protein S and antithrombin have been associated with worse sepsis outcomes [67]. To address this, various anticoagulant therapies have been tested to mitigate sepsis-associated coagulopathy, with limited success. Most recently, recombinant APC (Xigris, Eli Lilly) was shown to reduce mortality in severe sepsis patients and was licensed on this basis [68]. Subsequent trials, however, raised questions about the efficacy and safety of APC in this setting and Xigris was eventually voluntarily withdrawn for patient use [69]. Important anticoagulant receptors, such as thrombomodulin (TM) and the endothelial PC receptor (EPCR), are cleaved from the endothelial cell surface under inflammatory conditions. Soluble forms of both receptors are elevated in the plasma of individuals with sepsis [70]. Depleting endothelial cell surface EPCR and TM restricts PC activation, skewing the haemostatic balance towards thrombin generation. Interestingly, individuals with the rs2239562 single-nucleotide polymorphism in the promoter region of the thrombomodulin (*THBD)* gene have been suggested to be more susceptible to sepsis and experience worse outcomes [71].

Recent studies indicate that immune cross-talk has a role in the immunothrombotic state observed in malaria infection. Coagulation dysregulation is commonly observed among malaria patients and represents an often fatal complication of the disease [72]. A recent clinical trial revealed that healthy volunteers injected with low levels of *Plasmodium falciparum* sporozoites exhibited a hypercoagulable state. At detection of parasitaemia, the thrombin generation potential of plasma isolated from infected subjects was 17% higher compared with their baseline measurements [73]. In addition, malaria patient studies and preclinical malaria animal studies support the role of increased endothelial cell activation and UL-VWF secretion, in sequestering infected red blood cells in the microvasculature to promote cerebral vascular occlusion [74]. Taken together, these findings indicate that coagulopathy driven by acute infection arises from aberrant activation of multiple procoagulant mechanisms that are further amplified by inflammation-induced endothelial damage and diminished systemic anticoagulant activity.

The Coronavirus Disease in 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection represents an exemplar model of immunothrombotic disease driven by viral infection. VTE rates among COVID-19 patients are increased compared with healthy individuals [75,76], and COVID-19 causes a unique coagulopathy that can promote microvascular thromboses in the lung vasculature. Retrospective studies indicate that the severity of COVID-19 symptoms and the level of proinflammatory biomarkers in patients with COVID-19 positively correlated with the patient's risk of experiencing VTE [77]. For instance, in a dataset containing 6153 COVID-19 patients, only 0.09% of non-hospitalised COVID-19 patients developed VTE [77], whereas 3.1% of hospitalised patients experienced a VTE, rising to 7.2% of COVID-19 patients who required ventilation [77]. Similarly, in a cohort of 1114 COVID-19 patients, only a small number of thrombotic events were reported in COVID-19 outpatients, whilst the highest rates of thrombotic complications appeared in those COVID-19 patients admitted to ICU [78]. Numerous studies have highlighted potential mechanisms of prothrombotic activity

Clinical Science (2022) **136** 1809–1829
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associated with COVID-19 pathobiology. In severely ill patients, dysregulated inflammatory cytokine release promotes endotheliopathy and innate immune cell dysfunction, driving formation of a prothrombotic milieu [79]. Neutrophils play a critical role in COVID-19-associated coagulopathy. Plasma biomarkers of NET formation correlate with COVID-19 disease severity [80], and neutrophil-platelet aggregates may also contribute to enhanced platelet reactivity in COVID-19 patients [81,82]. Interestingly, patients with severe COVID-19 often exhibit thrombocytopenia, despite having increased generation of megakaryocytes and proplatelet formation. This disproportionate platelet count is likely due to thrombus deposition in multiple vessel beds [29,81].

A persistent immunothrombotic state in COVID-19 patients may also contribute to post-viral syndrome or 'long-COVID', a common feature in COVID-19 patients characterised by fatigue and chronic disease symptoms that can last for weeks and months post-infection [83]. The underlying cause of this fatigue is unclear but may arise in part due to damage to the endothelium arising from chronic hypoxia and inflammation [84]. In support of this, recent data indicate that individuals with 'long-COVID' exhibit a persistent endotheliopathy characterised by dysregulated VWF proteolysis, endothelial dysfunction and the persistence of activated monocyte and T-cell populations [85]. Anticoagulant and anti-inflammatory strategies targeting the inflamed endothelium may therefore provide a novel approach to mitigate symptoms associated with long COVID.

Does the adaptive immune system contribute to immunothrombosis?

Although adaptive immune system cells do not appear to contribute to normal haemostasis, recent work has highlighted a new role for T effector memory (T_{EM}) cells and regulatory T cells (Tregs) in modulating venous clot lysis. Unexpectedly, $CD44^{high}CD62L^{low}CD4^{+}$ and $CD8^{+}$ T_{EM} cells infiltrate the thrombus and vessel wall during DVT in a murine thrombosis model [86]. Infiltrating T_{EM} cells secrete IFNγ, independent of antigen stimulation, and recruit monocytes and neutrophils to the injury site, delaying thrombus resolution. Deficiency in T_{EM} cells enhanced thrombolysis and promoted increased matrix metalloproteinase-9 (MMP-9) expression by monocytes [86]. Previous studies have shown that suppression of MMP-9 in macrophages by T cell, NK cell and innate lymphoid cell-derived IFNγ delays clot resolution [87]. Tregs also play an unanticipated role in mediating efficient clot degradation, and their depletion results in delayed thrombolysis [88]. Furthermore, Treg population dynamics were found to influence monocyte recruitment and differentiation. Following TGFβ stimulation, a Treg subpopulation was found to produce secreted protein acidic and rich in cysteine (SPARC), a matricellular protein involved in cell turnover, tissue remodelling, and ECM repair. $SPARC^{+}$ Tregs were reported to recruit more CD11c+ monocytes to the thrombus with enhanced MMP and fibrinolytic activity [88]. Interestingly, cancer patients undergoing T cell targeted immunotherapies, such as immune checkpoint inhibitors (ICIs), exhibit an increased VTE risk (1.5- to 6.5-fold) [89] and cumulative incidence of VTE (5–10% at 12-month follow-up) [90], which may be due to the increased activity of effector T cells. These data demonstrate an unexpected but potentially important role for T cell sub-populations in modulating clot lysis and resolution.

Why do autoimmune disorders cause an increased VTE risk?

Current estimates report a global prevalence of autoimmune disease in 3–5% of the general population [91], with a recent report highlighting an increasing incidence of 3–9% per year [92]. Notably, increased VTE risk is characteristic of many autoimmune diseases, such as inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), and graft versus host disease (GvHD), and often represents a fatal disease complication.

IBD

IBD represents an expanding global healthcare burden affecting many adults and children. Approximately 0.3% of the worldwide population are diagnosed with IBD, with 25% of these cases arising in childhood and adolescents [93–95]. Notably, thromboembolic disease represents the most significant cause of mortality among IBD patients [96], with adult and paediatric IBD patients exhibiting a 3- and 6-fold increased risk of VTE development compared with the general population [97,98]. Yet, the mechanism(s) underlying this prothrombotic state remains unknown. Individuals with IBD are characterised by increased levels of proinflammatory immune cells, such as T cells, innate lymphoid cells, monocytes and macrophages, and cytokines, such as TNFα, IFNγ, IL-1β, IL-17a, IL-36α, IL-18, IL-6 and IL-8 [99–102]. Several plasma procoagulant and anti-fibrinolytic proteins, including PAI-1, fibrinogen, and prothrombin, are elevated during an IBD 'flare', in which symptoms are acutely increased [103–106].

In murine IBD models, TF inhibition is associated with reduced immunothrombotic activity [107]. In addition, increased numbers of procoagulant TF^+ MPs are shed by platelets and leukocytes in IBD patients [108,109]. Interestingly, these MPs also can stimulate neutrophil NETosis [110,111]. As NET density correlates with disease severity [27], targeting NET formation and MP interactions may represent a potential strategy to mitigate against immunothrombosis in IBD patients. Platelet dysregulation and hyperactivity also contribute to the prothrombotic state observed in IBD. Increased platelet activation in IBD patients has been observed, with platelet activation status correlating with disease severity [112,113].

Whereas chronic inflammation disrupts haemostatic balance to promote thrombosis, the ability of many coagulation proteases to modulate cell function, particularly the inflammatory response directly via cell signalling, suggests an additional mechanism by which aberrant coagulation protease activation, or loss of protective anticoagulant signalling responses, can modulate the mucosal immune response. The PC pathway has been implicated as an important regulator of mucosal immunity, regulating the inflammatory responses in the endothelium, epithelium, and innate and adaptive immune cells [114,115]. Transgenic mice with low plasma PC (<5% normal levels) display a constitutively enhanced proinflammatory and prothrombotic phenotype [116], and in the context of IBD, have been shown to develop spontaneous colitis due to increased TNF-mediated intestinal barrier permeability [114]. Colitogenic mice display a reduced capacity for PC activation [115] likely due to decreased EPCR and TM expression in the inflamed microvasculature [114,115]. Accordingly, EPCR-deficient mice exhibit exacerbated disease in DSS-induced colitis compared to wild-type mice [117]. Chronic inflammation may contribute to the increased VTE risk experienced by IBD patients. Interestingly, IBD patients receiving anti-TNF biologics exhibit a reduced risk of VTE compared with those on traditional corticosteroids [118], implicating TNFD-mediated inflammation as a critical feature of increased VTE risk in IBD patients. In contrast, however, other targeted immunobiologics, including ustekinumab, a monoclonal antibody targeting the p40 subunit of IL-12/23, and vedolizumab, a monoclonal antibody targeting the $\alpha_3\beta_7$ integrin, confer a potential risk of severe cardiovascular events to patients, including stroke [119], thrombocytopenia [120] and reduced clot formation time [121].

Graft versus host disease

Incidence of VTE among haematopoietic stem cell transplant (HSCT) patients ranges from 0.5 to 13%, and the occurrence of VTE is typically associated with the development of Graft versus Host Disease (GvHD) [122–129]. In those who develop VTE post-transplant, there is a 65% incidence in GvHD [122]. Furthermore, there is a higher risk of other thrombotic events associated with GvHD severity and donor blood group, with non-O-donor to recipient ABO match exhibiting a 2.7-fold higher hazard ratio of thrombotic complication compared to O-donor to O-recipient match [130]. Transplant-associated thrombotic microangiopathy (TA-TMA) is a common complication of HSCT and is associated with complement activation following an endothelial injury during GvHD [131]. The association between severity of inflammation exposure and risk of thrombosis in GvHD patients is exemplified by the lowered threshold for neutrophil NETosis in GvHD patients and the consequent role in increased plasma hypercoagulability. Paediatric recipients post-HSCT have elevated levels of plasma double-stranded DNA (dsDNA), a surrogate marker for NETs, alongside an increased risk of TA-TMA, onset of gastrointestinal GvHD and mortality [132].

SLE

VTE is a common co-morbidity in patients with systemic lupus erythematosus (SLE), and the risk of its development is commonly associated with the presence of antiphospholipid antibodies (APLA). To date, the most critical APLAs identified to confer thrombotic risk are anticardiolipin antibodies (ACA), lupus anticoagulant (LAC), and anti-β2-glycoprotein I (anti-β2-GPI) [133,134]. Despite this, 40% of SLE patients diagnosed with thrombotic complications are negative for APLAs [133], indicating a multifactorial mechanism for VTE in these patients. SLE patients display endothelial dysfunction, characterised by increased expression of fibrinogen and cell adhesion molecules, ICAM, VCAM, VEGF, and VWF [135].

Furthermore, SLE patients exhibit a reduced capacity to clear neutrophil NETs [136] and an enhanced adaptive proinflammatory cytokine and immune cell profile [137]. Interestingly, β2GPI-reactive $CD4^+$ T cells are hypothesised to drive auto-antibody production in SLE [138], thus amplifying the immunothrombotic environment. Recent work has identified an EPCR-lysophosphatidic acid (LBPA) complex expressed on monocyte cell surfaces as a receptor for APLAs that facilitates their internalisation and subsequent proinflammatory and procoagulant activities [139]. APLAs binding to EPCR-LBPA activate TF-FVIIa-FXa-PAR2 signalling via acid sphingomyelinase TF decryption to promote coagulation. APLA-mediated TF activation also induces IFN production from DCs, promoting the expansion of APLA-producing B1a cells, creating an immunothrombotic loop. Studies using EPCR-deficient transgenic

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

mice and EPCR-blocking antibodies revealed that EPCR-LBPA deficiency protects against APLA-induced thrombosis and foetal loss, murine SLE and APS [139]. Therefore, therapeutic targeting of this pathway holds promise in regulating APLA autoimmunity and thrombosis.

Allergies

Dysregulation of haemostatic and fibrinolytic activity is evident in many allergic diseases, including chronic spontaneous urticaria (CSU), asthma and dermatitis [140–143]. Still, the inflammatory mechanisms underlying this haemostatic dysregulation, such as elevated soluble TF levels, PAI-1 and thrombin activatable fibrinolysis inhibitor (TAFI) [143] found in asthma patient sputum, are poorly understood. Interestingly, there is a significantly increased risk of pulmonary embolism in asthma patients compared to the general population, linked to the frequency of asthma exacerbation and hospitalisation [144]. In the airways, TF expression is induced by IL-13, one of the major cytokines involved in asthma pathogenesis [145]. In parallel, FX is also implicated in airway dysfunction, and FXa signalling increases airway fibroblast proliferation via the production of autocrine platelet-derived growth factor (PDGF) and procollagen synthesis through PAR1 activation, resulting in airway fibrosis and reduced lung compliance. Accordingly, FXa inhibition with fondaparinux has been shown to alleviate airway pathology in a mouse asthma model [146,147]. Enhanced thrombin activation and reduced levels of APC are also reported in asthma patients [148–150]. Platelet hyperactivity and fibrin deposition all exacerbate asthma, contributing to bronchial hyperresponsiveness, constriction, airway remodelling and inflammation [151]. Similarly, fibrin degradation products (FDPs) contribute to airway dysregulation, promoting leukocyte infiltration, proinflammatory cytokine and PAI-1 release [151–154].

Allergies in the skin, such as atopic dermatitis and contact dermatitis, are chronic inflammatory disorders of the epidermis, affecting up to 10% of adults and 20% of children globally [155]. These conditions are triggered by skin exposure to potential allergens [156], which can induce an exaggerated T cell-mediated immune response [157]. The infiltrating T cells exert cytotoxic effector mechanisms on nearby keratinocytes, thus disrupting the skin barrier and allowing the further invasion of allergens [157]. Like airway inflammation, increased myeloid cell TF expression and activity are associated with skin allergy pathogenesis, and monocytes isolated from CSU patients express significantly more TF activity than healthy controls [158]. Coagulation proteases can also induce degranulation of human skin mast cells and basophils via complement C5a and C5aR, causing edema [159]. Similarly, thrombin and fibrinogen have been shown to play a pathological role in contact dermatitis. Children with contact dermatitis show a correlation between disease severity, transepidermal water loss (TEWL), and total thrombin generation. Inhibition of either thrombin or fibrinogen in preclinical dermatitis models alleviates disease [160], as does treatment with recombinant APC [142]. APC signalling through PAR2 helps clinical signs of disease by inhibiting CD4+ T cell generation and proinflammatory responses [142]. Collectively, these studies highlight an emerging role for dysregulated haemostatic protease signalling function in the development and persistence of allergic skin disease.

Neurodegenerative disease and ageing

Aberrant coagulation pathway activity and signalling have been implicated in the pathogenesis of several neurological disorders. Multiple sclerosis (MS) is an immune-mediated demyelinating and degenerative disease of the central nervous system (CNS) [161]. MS patients have a 2-fold higher VTE risk compared with the general population [162]. Histopathological studies by Putnam in the 1930s reported an increased incidence of thrombi in plaques surrounding the CNS vasculature of MS patients that did not form in response to vessel wall injury but instead due to 'abnormal lability in blood plasma' [163,164]. Since then, MS patients have exhibited increased fibrin deposition around active MS lesions, impaired fibrinolytic activity, high soluble TF levels in the CNS, circulating soluble TM and elevated VWF activity reflective of endotheliopathy [165–169]. These studies indicate that proinflammatory and coagulation pathways are co-activated during MS pathogenesis, and dual targeting of both systems may be beneficial in this setting. In support of this, recent work selectively targeting a cryptic fibrin epitope ($\gamma_{377\text{-}395}$) with a monoclonal antibody (5B8) was beneficial in preventing amyloid-driven neurodegeneration and CNS autoimmunity in preclinical models of MS and Alzheimer's disease (AD) [170]. 5B8 inhibited fibrin binding to CR3, thus suppressing fibrin-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, subsequent reactive oxygen species (ROS) release and innate immune-driven neurotoxicity. 5B8 down-regulated expression of genes involved in immune cell recruitment and proinflammatory responses in fibrin-treated bone marrow-derived macrophages and reduced axonal damage, ROS generation, demyelination and $CD11b^+$ cell innate-driven inflammation in EAE [170]. Furthermore, inhibiting fibrin/CD11b interactions with 5B8 reduced loss of cholinergic neurons and microglia activation around plaques in 5XFAD mice and down-regulated critical pathways involved in neurodegeneration and oxidative stress, complement pathway, lysozyme, antigen presentation, cytokine response, and ROS pathway signalling. Significantly, this antibody

did not interfere with normal haemostasis, with no differences reported in fibrin polymerisation *in vitro*, clotting time, or partial thromboplastin time (aPTT) in human plasma treated with 5B8 reported compared to IgG2b-treated plasma controls [170].

Elevated levels of FXII have also been observed in the plasma of MS patients and may contribute to the thrombo-inflammatory state observed in the CNS of affected individuals [171,172]. EAE mice display significantly elevated FXII levels in the plasma, cerebrospinal fluid (CSF), and lymph nodes compared with control mice. FXII deficiency ameliorates disease via inhibition of DC-mediated priming of Th17 cell pathogenic responses [171]. Importantly, mechanistic studies have revealed that FXIIa inhibition inhibits thrombus formation but does not affect haemostasis [4,173,174]. Therefore, inhibiting contact activation may prove a safe method to break the immunothrombotic loop observed in neuro-inflammatory and neurogenerative diseases, without enhancing the risk of bleeding.

The risk of thrombosis increases with age. This is probably influenced by 'inflammaging', which describes the chronic low-grade and systemic inflammation that accelerates during biological ageing and occurs through various mechanisms, including immunosenescent cell generation, increased intestinal barrier permeability, bacterial dysbiosis, and leakage [175,176]. Collectively, these processes combine to induce increased levels of proinflammatory cytokines, chemokines, and immune cells in the vasculature, creating a lower threshold for pro-thrombotic events.

For example, older mice are reported to exhibit higher levels of TNFα in their plasma compared with young mice [177,178] and inflammaging contributes to age-related platelet hyperactivity via exposure to enhanced constitutive levels of proinflammatory cytokines such as TNF in the blood. Following stimulation, platelets from older mice also express increased levels of αIIbβ3 and PS and display an enhanced capacity to form thrombi *ex vivo*. Furthermore, TNF levels are heightened in the bone marrow compartment of older mice [179], suggesting local megakaryocytes will be more susceptible to inflammaging. Interestingly, monocytes have been proposed as the cellular source of TNF in this environment [180]. Monocyte-derived TNF is implicated in the increased platelet count and reactivity observed in older patients with myeloproliferative disorders [181,182]. Enhanced platelet–monocyte interactions result in proinflammatory cytokine production [183], which may be exacerbated by platelet derived-MPs that are also increased in older individuals [184].

Elevated fibrin levels have been observed in AD patient brains post-mortem, and signalling by this protein is linked to Aβ and tau deposition, vascular dysfunction, inflammation, and neurodegeneration [185]. In an open-label study of patients with mild to moderate AD, treatment with the thrombin inhibitor hirudin with donepezil significantly reduced the rate of cognitive decline among patients [186], suggesting that coagulation inhibition may be a novel strategy for mitigating neurodegenerative and neuroinflammatory disease. Furthermore, there is emerging evidence for the role of neutrophils and NETosis in AD pathology. Neutrophils, NETs and NET-associated MPO have been found in plaques surrounding the microvasculature in post-mortem AD patient brains and murine AD models [187,188]. The contact pathway is also implicated in AD pathogenesis. FXII has been detected in Aβ plaques [188], increased plasma kallikrein activity has been observed in the AD brain parenchyma [189], and enhanced levels of HK cleavage in the cerebrospinal fluid in AD patients compared with non-disease controls [190]. *In vitro* studies have found that Aβ can trigger FXIIa-dependent kallikrein activity and kallikrein-mediated cleavage of HK [191–193]. *In vivo* studies have supported these findings, suggesting Aβ plaques can mediate the upregulation of contact activation [194]. Notably, FXII inhibition was beneficial in AD mice, which displayed improved cognitive functioning compared with untreated controls. Furthermore, FXII depletion in AD mice resulted in diminished plasma HK cleavage and reduced fibrinogen deposition, neurodegeneration and neuroinflammation compared with untreated controls [195]. Collectively, these studies indicate that activation of the contact pathway contributes to the vascular and inflammatory dysfunction observed in AD.

Stress and circadian rhythm disruption

Stress is a risk factor for neurodegenerative disease [196,197], metabolic disease [198], cancer [199] and, with particular relevance to this review, thromboembolic disease [200]. Both acute and chronic stress causes a change in the distribution of immune cells between the circulation and tissue [201,202]. Assessment of the effect of stress on hypercoagulability is challenging, and several studies have generated disparate results. Following induction of acute stress by a public speaking task and a mock job interview, individuals exhibited increased plasma levels of D-dimer and VWF [203,204]. A comprehensive acute stress test study analysed multiple plasma coagulation parameters before and after stress. They found significant increases in FVII and FVIII activity and increased VWF and D-dimer levels in acutely stressed individuals compared to unstressed individuals. No significant changes were seen in fibrinogen and soluble TF levels [205]. These studies indicate that acute stress may promote transient changes in plasma hypercoagulability.

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

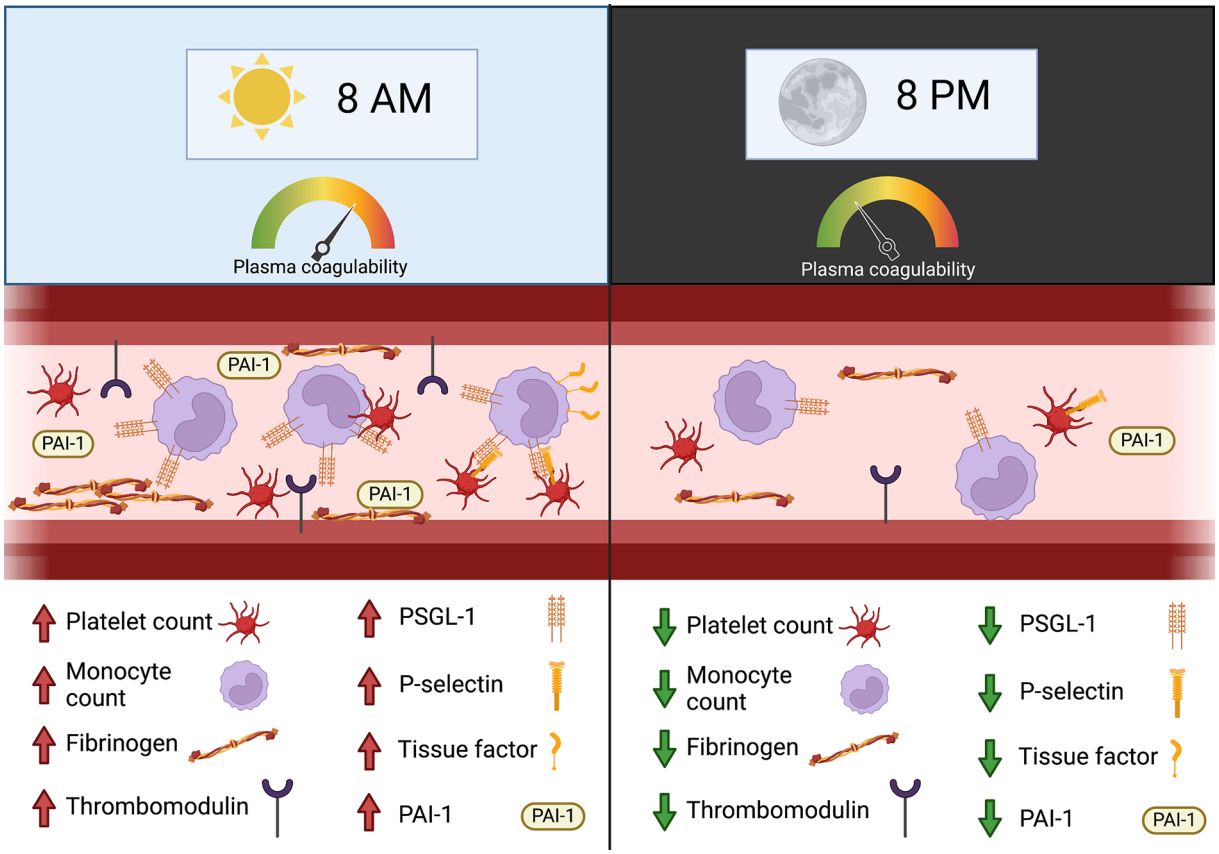

Figure 3. The role of circadian variation in modulating haemostatic activity

Levels of various procoagulant proteins, including fibrinogen and thrombomodulin, as well as fibrinolysis inhibitor PAI-1, vary with time of day, skewing the plasma and vessel surface environment in the morning towards procoagulant activity. Similarly, platelet and monocyte count increase in the morning, as is the expression of pro-adhesive cell surface receptors, such as PSGL-1. This can facilitate monocyte binding to platelet P-selectin to form aggregates, triggering increased TF activity on the monocyte surface. Collectively, circadian variation in plasma coagulation protein levels, innate immune cell number and activity, and vessel surface receptor composition may contribute to observed time-of-day effects on acute cardiovascular disease manifestation.

However, more extensive studies are required to assess the mechanistic basis for these biological changes and their potential long-term impact on other haemostatic parameters [206].

According to the 2021 edition of 'Living and working in Europe' [207] 19% of the EU population worked at least once during the night every month, and 21% were shift workers. Other sources of circadian disruption are social and travel-related jet lag, as well as light pollution. Circadian disruption is now well-established to disrupt normal immune cell function, and emerging data suggest a shared role in the dysregulation of haemostasis (Figure 3). The rhythmic expression of genes associated with coagulation may also contribute to the increased incidence of acute cardiovascular disease in the morning hours of the day [208,209].

Circadian rhythms are controlled by a transcriptional-translational feedback loop present in almost all cell types (for detailed review, see [210]). The positive arm of the loop is controlled by transcription factors BMAL1 and CLOCK, which heterodimerise and drive the transcription of *Per* and *Cry* genes. Once phosphorylated, PER and CRY can re-enter the nucleus and act as transcriptional repressors of the BMAL1/CLOCK heterodimer, making up the negative arm of the loop. This prevents transcription of *Per* and *Cry*, reducing production of the repressors and allowing BMAL1/CLOCK to function again. Another part of the loop is formed by RORα and REV-ERBα. Their transcription is under the control of BMAL1/CLOCK and they drive or repress *Bmal1* transcription, respectively. BMAL1 and CLOCK are active during daytime, while PER, CRY, RORα and REV-ERBα are active during night-time. The complete feedback loop takes 24 h to complete, giving rhythmicity to the cell and any genes under the control of these clock transcription factors, termed clock-controlled genes (CCGs). BMAL1/CLOCK bind to E-box sequences while RORα and REV-ERBα bind to RORE sequences.

Bmal1$^{-/-}$ mice age prematurely and lack the characteristic rhythmic expression of clock target genes [211]. Notably, several coagulation and haemostasis-related genes are CCGs, including *Serpine1* [212], *F7* [213,214], *F12* [214] *and Thbd* [215]. *Bmal1*$^{-/-}$ mice exhibit a prothrombotic phenotype and clot more rapidly in a $FeCl_3$ treatment model [216]. A shifted light schedule in mice also decreased prothrombin time, plasma fibrinogen concentration, and increased bleeding time, illustrating how coagulation changes can be driven by circadian rhythm disruption [214]. It is important to consider that mice and humans exhibit opposing circadian schedules, where mice are nocturnal and humans are diurnal, making human studies important for fully elucidating the role of circadian rhythms in coagulation. In humans, PAI-1, tPA and fibrinogen concentrations in the blood vary over 24 h [217]. PAI-1 is subject to diurnal fluctuation, independent of any day- or night-time associated behaviours [218], suggesting the capacity for thrombus formation and clearance will vary with time of day. Mouse and human neutrophils have been shown different propensities to form NETs based on time of day [219]. Mouse neutrophils isolated at night-time form more NETs than day-time neutrophils, while the opposite is seen in human neutrophils (in keeping with their opposite circadian rhythms [219]. Inflammatory (Ly6C^{hi}) monocytes also contribute to venous thrombus formation [220] and their number is also subject to diurnal oscillations [221]. Platelet aggregation and interactions between neutrophils and monocytes is an integral arm of immunothrombosis, and their activity and distribution are also circadian. Platelet expression of activation surface markers, including GPIIb-IIIa, GpIb and P-selectin, is highest in the morning (8–9 AM). In contrast, the threshold for platelet aggregation is lowest in the evening (8 PM) [222]. Interestingly, the efficacy of the platelet inhibitor Ticagrelor (Brilinta, AstraZeneca) also varies modestly with time of day, with the lowest effectiveness at 1 PM [223]. Circadian nuclear receptor Rev-Erbα has recently been shown to have a role in platelet activation and aggregation [224]. Platelet-specific deletion of Rev-Erbα in mice and pharmacological inhibition of Rev-Erbα in human platelets showed decreased activation and aggregation by light aggregometry assays compared with untreated platelets [224]. The proposed mechanism involves GTPase activating protein Oligophrenin-1 (OPHN1), which is protected from degradation following binding to Rev-Erbα in platelets [224] and the brain [225]. Activated OPHN1 binds and activates GTPase RhoA, which is required for actin remodelling upon platelet activation [226]. Furthermore, mice whose circadian rhythms were disrupted were found to develop larger atherosclerotic lesions using a murine atherosclerotic model in which the mice were exposed to a reversed light/dark schedule. Subsequently, it was found that *Bmal1* expression is altered in the foam cells in those lesions, and foam cells experience increased ER stress compared with mice in normal light conditions [227]. Collectively, these studies highlight the variance in clotting proteins and cellular components at different times of day. These studies also highlight a potential role for time of day in the effectiveness of anticoagulant and antithrombotic drugs.

New anti-immunothrombotic therapies? APC beyond sepsis

APC demonstrates potent anti-inflammatory and cytoprotective signalling properties on multiple cell types [115,228–235]. Nevertheless, APC signalling primarily occurs via activation of protease-activated receptor 1 (PAR1) when APC when bound to the EPCR [236]. Despite the failure of recombinant APC (Xigris, Eli Lily) in the treatment of severe sepsis, multiple studies have demonstrated that APC cytoprotective and anti-coagulant mechanisms can be targeted independently [237], and a variety of APC mutants altered to possess no anticoagulant activity but with cytoprotective signalling-specific activity could prove therapeutically beneficial in several inflammatory disease states. APC has been shown in many preclinical models to modulate thrombo-inflammation via regulation of both innate and adaptive immune cell signalling. For example, administration of exogenous APC ameliorates disease and induces mucosal healing by promoting barrier integrity, inhibiting leukocyte adhesion, a proinflammatory cytokine, and chemokine release in murine colitis models [114,115]. APC administration to a preclinical mouse model of asthma significantly attenuated airway inflammation via decreased neutrophil, DC and eosinophil influx, reduced type 2 cytokine levels, and IgE concentration in BAL fluid. Interestingly, these effects occurred independently of APC anticoagulant activity, indicative of a critical role for its signalling function [232,238,239]. Attempts to treat airway inflammation via FXa inhibition or by limiting TF activity resulted in severe bleeding episodes or haemorrhagic events [240–242], highlighting the challenge of traditional anticoagulant strategies to restrain the proinflammatory signalling activity of procoagulant proteases. In contrast, non-anticoagulant, signalling-selective recombinant APC variants may represent a useful strategy to address allergic airway inflammation. Administration of recombinant APC to DCs *in vitro* alters their population dynamics, shifting the cells from a proinflammatory conventional DC phenotype towards an anti-inflammatory plasmacytoid DC phenotype while simultaneously reducing IL-6 and IL-10 secretion [239]. As such, APC-mediated DC reprogramming renders these cells less effective in priming Th1 and

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

Th2 cells *in vitro* and diminished hyperresponsiveness in an allergic airway challenge *in vivo*. Mice adoptively transferred with APC-treated ovalbumin-pulsed DCs exhibited significantly decreased lung concentrations of Th1 and Th2 cytokines, IFNγ and IL-5, respectively, and lower levels of immunoglobulin E (IgE) in the plasma [239].

APC can also regulate the differentiation and pathogenicity of Th17 cells. Administration of recombinant APC or PROCR deficiency inhibits the generation of Th17 cells *in vitro* and ameliorates Th17 mediated EAE *in vivo* [165,243]. Aside from inhibiting proinflammatory T-cell responses, APC also enhances anti-inflammatory Treg generation and suppressive functioning. Interestingly, administration of recombinant APC ameliorates disease in MS-like experimental autoimmune encephalomyelitis (EAE) [165,168] and APC mutants with either cytoprotective or anticoagulant mechanisms both alleviated EAE disease in mice [165]. Similarly, 3K3A-APC, a recombinant non-anticoagulant APC mutant, exerts therapeutic benefits in clinical models of AD, with reduced Aβ deposition, improved cerebrovascular integrity, and diminished neuroinflammatory responses reported [244]. 3K3A-APC also promotes BBB integrity and prevents neuronal damage during ischemic stroke in pericyte-deficient mice [228]. These studies indicate that APC treatment may benefit neurological conditions associated with pericyte loss, such as AD, dementia, and ageing.

In murine GvHD, pre-incubation of pan T-cell populations with APC before transplantation prevented the induction of disease via PAR-2/PAR-3 signalling, resulting in significantly lower levels of IFNγ, TNFα, and IL-17a plasma cytokines and reduced accumulation of IFNγ, TNFα, and IL-17^{+}CD4^{+} splenic T cells when compared to non-APC treated T cell-recipient mice. Furthermore, APC-treated mice exhibited an enhanced regulatory phenotype with significantly increased numbers of FOXP3^{+}CD4^{+} Treg cells in the periphery, alongside elevated levels of TGFβ and IL-10 in the plasma, compared with control mice. Mechanistic studies *in vitro* revealed a similar trend in human T cells, with APC incubation enhancing Treg generation and expansion, inhibiting Th1 and Th17 differentiation [233]. These studies indicate the potential use of APC in T cell immunotherapy, inhibiting T cell-mediated pathology and associated immunothrombotic effects.

Conclusions

Proinflammatory pathways overlap with and trigger many prothrombotic molecular and cellular activities, including myeloid cell activation, neutrophil NETosis, enhanced platelet reactivity and the release of procoagulant microparticles. Although well-established in sepsis, increased thrombogenicity is increasingly recognised as a common feature in disparate acute and chronic inflammatory disease settings. In addition, dysregulation of immunity caused by standard features of modern lifestyles, such as chronic stress and circadian disruption also unbalances haemostasis and understanding the biological processes underlying these changes represents a significant new challenge. Similarly, whereas an increased risk of VTE in the elderly is well-established, the growing appreciation that this may arise as a by-product of 'inflammaging' epitomises the emerging paradigm of immunothrombosis. Despite the increasing recognition of the prothrombotic nature of inflammatory disease, significant challenges remain in managing affected patients and new therapeutic approaches to curtail the key pathogenic steps described are urgently required.

Data Availability

NA

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

Grant support (to R.J.S.P.) is provided by Science Foundation Ireland [grant numbers 20/COV/8511 and 21/FFP- A/8859] and the National Children's Research Centre [grant numbers C/18/3 and PRPG/H/18/315].

CRediT Author Contribution

Paula A. Klavina: Conceptualization, Writing—original draft, Writing—review & editing. **Gemma Leon:** Conceptualization, Writing—original draft, Writing—review & editing. **Annie M. Curtis:** Conceptualization, Writing—original draft, Writing—review & editing. **Roger J.S. Preston:** Conceptualization, Writing—original draft, Writing—review & editing.

Abbreviations

AD, Alzheimer's disease; APC, activated protein C; CLR, C-type lectin receptor; CSF, cerebrospinal fluid; DC, dendritic cell; DVT, deep vein thrombosis; EAE, experimental autoimmune encephalomyelitis; IgE, immunoglobulin E; NLR, Nod-like receptor;

Nod, nucleotide-binding oligomerization domain; PAR1, protease-activated receptor 1; PRR, pathogen recognition receptor; PSGL-1, P-selectin glycoprotein ligand 1; RLR, RIG I-like receptor; ROS, reactive oxygen species; TAFI, thrombin activatable fibrinolysis inhibitor; TF, tissue factor; TFPI, tissue factor pathway inhibitor; tPA, tissue-type plasminogen activator; VTE, venous thromboembolism; VWF, von Willebrand factor.

References

1 O'Donnell, J.S., O'Sullivan, J.M. and Preston, R.J.S. (2019) Advances in understanding the molecular mechanisms that maintain normal haemostasis. *Br. J. Haematol.* **186**, 24–36, https://doi.org/10.1111/bjh.15872
2 Vu, T.-K.H., Hung, D.T., Wheaton, V.I. and Coughlin, S.R. (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057–1068, https://doi.org/10.1016/0092-8674(91)90261-V
3 Preston, R.J.S., O'Sullivan, J.M. and O'Donnell, J.S. (2019) Advances in understanding the molecular mechanisms of venous thrombosis. *Br. J. Haematol.* **186**, 13–23, https://doi.org/10.1111/bjh.15869
4 Heestermans, M., Naudin, C., Mailer, R.K., Konrath, S., Klaetschke, K., Jämsä, A. et al. (2021) Identification of the factor XII contact activation site enables sensitive coagulation diagnostics. *Nat. Commun.* **12**, 5596, https://doi.org/10.1038/s41467-021-25888-7
5 Grover, S.P. and Mackman, N. (2019) Intrinsic pathway of coagulation and thrombosis. *Arterioscler. Thromb. Vasc. Biol.* **39**, 331–338, https://doi.org/10.1161/ATVBAHA.118.312130
6 Morrissey, J.H., Choi, S.H. and Smith, S.A. (2012) Polyphosphate: an ancient molecule that links platelets, coagulation, and inflammation. *Blood* **119**, 5972–5979, https://doi.org/10.1182/blood-2012-03-306605
7 Müller, F., Mutch, N.J., Schenk, W.A., Smith, S.A., Esterl, L., Spronk, H.M. et al. (2009) Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. *Cell* **139**, 1143–1156, https://doi.org/10.1016/j.cell.2009.11.001
8 Iwasaki, A. and Medzhitov, R. (2015) Control of adaptive immunity by the innate immune system. *Nat. Immunol.* **16**, 343–353, https://doi.org/10.1038/ni.3123
9 von Moltke, J., Ayres, J.S., Kofoed, E.M., Chavarría-Smith, J. and Vance, R.E. (2013) Recognition of bacteria by inflammasomes. *Annu. Rev. Immunol.* **31**, 73–106, https://doi.org/10.1146/annurev-immunol-032712-095944
10 Gürtler, C. and Bowie, A.G. (2013) Innate immune detection of microbial nucleic acids. *Trends Microbiol.* **21**, 413–420, https://doi.org/10.1016/j.tim.2013.04.004
11 Yoneyama, M., Onomoto, K., Jogi, M., Akaboshi, T. and Fujita, T. (2015) Viral RNA detection by RIG-I-like receptors. *Curr. Opin. Immunol.* **32**, 48–53, https://doi.org/10.1016/j.coi.2014.12.012
12 Rathinam, V.A.K. and Fitzgerald, K.A. (2011) Cytosolic surveillance and antiviral immunity. *Curr. Opin. Virol.* **1**, 455–462, https://doi.org/10.1016/j.coviro.2011.11.004
13 Thomas, C.J. and Schroder, K. (2013) Pattern recognition receptor function in neutrophils. *Trends Immunol.* **34**, 317–328, https://doi.org/10.1016/j.it.2013.02.008
14 Igyártó, B.Z., Haley, K., Ortner, D., Bobr, A., Gerami-Nejad, M., Edelson, B.T. et al. (2011) Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. *Immunity* **35**, 260–272, https://doi.org/10.1016/j.immuni.2011.06.005
15 Mashayekhi, M., Sandau, M.M., Dunay, I.R., Frickel, E.M., Khan, A., Goldszmid, R.S. et al. (2011) CD8α(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by toxoplasma gondii tachyzoites. *Immunity* **35**, 249–259, https://doi.org/10.1016/j.immuni.2011.08.008
16 Edelson, B.T., Bradstreet, T.R., Hildner, K., Carrero, J.A., Frederick, K.E., Kc, W. et al. (2011) CD8α(+) dendritic cells are an obligate cellular entry point for productive infection by listeria monocytogenes. *Immunity* **35**, 236–248, https://doi.org/10.1016/j.immuni.2011.06.012
17 Edelson, B.T., Bradstreet, T.R., Kc, W., Hildner, K., Herzog, J.W., Sim, J. et al. (2011) Batf3-dependent CD11b(Low/-) peripheral dendritic cells are GM-CSF-independent and are not required for Th cell priming after subcutaneous immunization. *PLoS ONE* **6**, e25660, https://doi.org/10.1371/journal.pone.0025660
18 Hernández-Santos, N. and Gaffen, S.L. (2012) Th17 cells in immunity to *Candida albicans*. *Cell Host Microbe* **11**, 425–435, https://doi.org/10.1016/j.chom.2012.04.008
19 Romani, L. (2008) Cell mediated immunity to fungi: a reassessment. *Med. Mycol.* **46**, 515–529, https://doi.org/10.1080/13693780801971450
20 Murakami, R., Denda-Nagai, K., Hashimoto, S., Nagai, S., Hattori, M. and Irimura, T. (2013) A unique dermal dendritic cell subset that skews the immune response toward Th2. *PLoS ONE* **8**, e73270, https://doi.org/10.1371/journal.pone.0073270
21 Kumamoto, Y., Linehan, M., Weinstein, J.S., Laidlaw, B.J., Craft, J.E. and Iwasaki, A. (2013) CD301b$^+$ dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity* **39**, 733–743, https://doi.org/10.1016/j.immuni.2013.08.029
22 Gao, Y., Nish, S.A., Jiang, R., Hou, L., Licona-Limón, P., Weinstein, J.S. et al. (2013) Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* **39**, 722–732, https://doi.org/10.1016/j.immuni.2013.08.028
23 del Rio, M.-L., Rodriguez-Barbosa, J.-I., Kremmer, E. and Förster, R. (2007) CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T Cells. *J. Immunol. Baltim. Md 1950* **178**, 6861–6866
24 Hildner, K., Edelson, B.T., Purtha, W.E., Diamond, M., Matsushita, H., Kohyama, M. et al. (2008) Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* **322**, 1097–1100, https://doi.org/10.1126/science.1164206
25 Edelson, B.T., Kc, W., Juang, R., Kohyama, M., Benoit, L.A., Klekotka, P.A. et al. (2010) Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J. Exp. Med.* **207**, 823–836, https://doi.org/10.1084/jem.20091627
26 Castanheira, F.V.S. and Kubes, P. (2019) Neutrophils and NETs in modulating acute and chronic inflammation. *Blood* **133**, 2178–2185, https://doi.org/10.1182/blood-2018-11-844530

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

27 Schroder, A.L., Chami, B., Liu, Y., Doyle, C.M., El Kazzi, M., Ahlenstiel, G. et al. (2022) Neutrophil extracellular trap density increases with increasing histopathological severity of Crohn's disease. *Inflamm. Bowel Dis.* **28**, 586–598, https://doi.org/10.1093/ibd/izab239

28 Wolach, O., Sellar, R.S., Martinod, K., Cherpokova, D., McConkey, M., Chappell, R.J. et al. (2018) Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci. Transl. Med.* **10**, eaan8292, https://doi.org/10.1126/scitranslmed.aan8292

29 Boeckh-Behrens, T., Golkowski, D., Ikenberg, B., Schlegel, J., Protzer, U., Schulz, C. et al. (2021) COVID-19-associated large vessel stroke in a 28-year-old patient: NETs and platelets possible key players in acute thrombus formation. *Clin. Neuroradiol.* **31**, 511–514, https://doi.org/10.1007/s00062-020-00992-1

30 Martinod, K. and Wagner, D.D. (2014) Thrombosis: tangled up in NETs. *Blood* **123**, 2768–2776, https://doi.org/10.1182/blood-2013-10-463646

31 Moschonas, I.C. and Tselepis, A.D. (2019) The pathway of neutrophil extracellular traps towards atherosclerosis and thrombosis. *Atherosclerosis* **288**, 9–16, https://doi.org/10.1016/j.atherosclerosis.2019.06.919

32 Massberg, S., Grahl, L., von Bruehl, M.-L., Manukyan, D., Pfeiler, S., Goosmann, C. et al. (2010) Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat. Med.* **16**, 887–896, https://doi.org/10.1038/nm.2184

33 Jiménez-Alcázar, M., Rangaswamy, C., Panda, R., Bitterling, J., Simsek, Y.J., Long, A.T. et al. (2017) Host DNases prevent vascular occlusion by neutrophil extracellular traps. *Science* **358**, 1202–1206, https://doi.org/10.1126/science.aam8897

34 Semeraro, F., Ammollo, C.T., Morrissey, J.H., Dale, G.L., Friese, P., Esmon, N.L. et al. (2011) Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* **118**, 1952–1961, https://doi.org/10.1182/blood-2011-03-343061

35 Yang, J., Wu, Z., Long, Q., Huang, J., Hong, T., Liu, W. et al. (2020) Insights into immunothrombosis: the interplay among neutrophil extracellular trap, von Willebrand Factor, and ADAMTS13. *Front. Immunol.* **11**, https://doi.org/10.3389/fimmu.2020.610696

36 Ammollo, C.T., Semeraro, F., Xu, J., Esmon, N.L. and Esmon, C.T. (2011) Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation: histones impair TM-dependent protein C activation. *J. Thromb. Haemost.* **9**, 1795–1803, https://doi.org/10.1111/j.1538-7836.2011.04422.x

37 Kawalkowska, J., Quirke, A.-M., Ghari, F., Davis, S., Subramanian, V., Thompson, P.R. et al. (2016) Abrogation of collagen-induced arthritis by a peptidyl arginine deiminase inhibitor is associated with modulation of T cell-mediated immune responses. *Sci. Rep.* **6**, 26430, https://doi.org/10.1038/srep26430

38 Shahneh, F., Christian Probst, H., Wiesmann, S.C., A-Gonzalez, N., Ruf, W., Steinbrink, K. et al. (2022) Inflammatory monocyte counts determine venous blood clot formation and resolution. *Arterioscler. Thromb. Vasc. Biol.* **42**, 145–155, https://doi.org/10.1161/ATVBAHA.121.317176

39 Egorina, E.M., Sovershaev, M.A., Bjørkøy, G., Gruber, F.X.E., Olsen, J.O., Parhami-Seren, B. et al. (2005) Intracellular and surface distribution of monocyte tissue factor. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1493–1498, https://doi.org/10.1161/01.ATV.0000168413.29874.d7

40 von Brühl, M.-L., Stark, K., Steinhart, A., Chandraratne, S., Konrad, I., Lorenz, M. et al. (2012) Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J. Exp. Med.* **209**, 819–835, https://doi.org/10.1084/jem.20112322

41 Rudd, K.E., Johnson, S.C., Agesa, K.M., Shackelford, K.A., Tsoi, D., Kievlan, D.R. et al. (2020) Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. *Lancet North Am. Ed.* **395**, 200–211, https://doi.org/10.1016/S0140-6736(19)32989-7

42 Álvaro-Meca, A., Jiménez-Sousa, M.A., Micheloud, D., Sánchez-Lopez, A., Heredia-Rodríguez, M., Tamayo, E. et al. (2018) Epidemiological Trends of Sepsis in the Twenty-First Century (2000-2013): An Analysis of Incidence, Mortality, and Associated Costs in Spain. *Popul. Health Metr.* **16**, 4, https://doi.org/10.1186/s12963-018-0160-x

43 Kumar, G., Kumar, N., Taneja, A., Kaleekal, T., Tarima, S., McGinley, E. et al. (2011) Nationwide trends of severe sepsis in the 21st century (2000-2007). *Chest* **140**, 1223–1231, https://doi.org/10.1378/chest.11-0352

44 Healy, M. (2020) National sepsis report 2019. https://www.hse.ie/eng/about/who/cspd/ncps/sepsis/news/national-sepsis-report-2019.pdf

45 Gyawali, B., Ramakrishna, K. and Dhamoon, A.S. (2019) Sepsis: the evolution in definition, pathophysiology, and management. *SAGE Open Med.* **7**, 2050312119835043, https://doi.org/10.1177/2050312119835043

46 Opal, S.M. and van der Poll, T. (2015) Endothelial barrier dysfunction in septic shock. *J. Intern. Med.* **277**, 277–293, https://doi.org/10.1111/joim.12331

47 Saito, S., Uchino, S., Hayakawa, M., Yamakawa, K., Kudo, D., Iizuka, Y. et al. (2019) Epidemiology of disseminated intravascular coagulation in sepsis and validation of scoring systems. *J. Crit. Care* **50**, 23–30, https://doi.org/10.1016/j.jcrc.2018.11.009

48 Gando, S., Shiraishi, A., Yamakawa, K., Ogura, H., Saitoh, D., Fujishima, S. et al. (2019) Role of disseminated intravascular coagulation in severe sepsis. *Thromb. Res.* **178**, 182–188, https://doi.org/10.1016/j.thromres.2019.04.025

49 Ko, B.S., Cho, H.Y., Ryoo, S.M., Kim, M.C., Jung, W., Park, S.H. et al. (2016) The prevalence and significance of overt disseminated intravascular coagulation in patients with septic shock in the emergency department according to the third international consensus definition. *Korean J. Crit. Care Med.* **31**, 334–341, https://doi.org/10.4266/kjccm.2016.00339

50 Taylor, F.B., Chang, A., Ruf, W., Morrissey, J.H., Hinshaw, L., Catlett, R. et al. (1991) Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. *Circ. Shock* **33**, 127–134

51 Creasey, A.A., Chang, A.C., Feigen, L., Wün, T.C., Taylor, F.B. and Hinshaw, L.B. (1993) Tissue factor pathway inhibitor reduces mortality from *Escherichia coli* septic shock. *J. Clin. Invest.* **91**, 2850–2860, https://doi.org/10.1172/JCI116529

52 Levi, M., ten Cate, H., Bauer, K.A., van der Poll, T., Edgington, T.S., Büller, H.R. et al. (1994) Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. *J. Clin. Invest.* **93**, 114–120, https://doi.org/10.1172/JCI116934

53 Zwicker, J.I., Trenor, C.C., Furie, B.C. and Furie, B. (2011) Tissue factor-bearing microparticles and thrombus formation. *Arterioscler. Thromb. Vasc. Biol.* **31**, 728–733, https://doi.org/10.1161/ATVBAHA.109.200964

54 Wu, C., Lu, W., Zhang, Y., Zhang, G., Shi, X., Hisada, Y. et al. (2019) Inflammasome activation triggers blood clotting and host death through pyroptosis. *Immunity* **50**, 1401.e4–1411.e4, https://doi.org/10.1016/j.immuni.2019.04.003
55 Yang, X., Cheng, X., Tang, Y., Qiu, X., Wang, Y., Kang, H. et al. (2019) Bacterial endotoxin activates the coagulation cascade through gasdermin D-dependent phosphatidylserine exposure. *Immunity* **51**, 983.e6–996.e6, https://doi.org/10.1016/j.immuni.2019.11.005
56 Zhang, Y., Cui, J., Zhang, G., Wu, C., Abdel-Latif, A., Smyth, S.S. et al. (2021) Inflammasome activation promotes venous thrombosis through pyroptosis. *Blood Adv.* **5**, 2619–2623, https://doi.org/10.1182/bloodadvances.2020003041
57 Denorme, F., Vanhoorelbeke, K. and De Meyer, S.F. (2019) Von Willebrand factor and platelet glycoprotein Ib: a thromboinflammatory axis in stroke. *Front. Immunol.* **10**, 2884, https://doi.org/10.3389/fimmu.2019.02884
58 Levy, G.G., Nichols, W.C., Lian, E.C., Foroud, T., McClintick, J.N., McGee, B.M. et al. (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* **413**, 488–494, https://doi.org/10.1038/35097008
59 Pendu, R., Terraube, V., Christophe, O.D., Gahmberg, C.G., de Groot, P.G., Lenting, P.J. et al. (2006) P-selectin glycoprotein ligand 1 and β2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. *Blood* **108**, 3746–3752, https://doi.org/10.1182/blood-2006-03-010322
60 Rautiainen, L., Cirko, A., Pavare, J., Balmaks, R., Grope, I., Katirlo, I. et al. (2019) Assessment of ADAMTS-13 level in hospitalized children with serious bacterial infections as a possible prognostic marker. *Medicina (Mex)* **55**, 503, https://doi.org/10.3390/medicina55080503
61 Smeets, N.J.L., Fijnheer, R., Sebastian, S. and De Mast, Q. (2018) Secondary thrombotic microangiopathy with severely reduced ADAMTS13 activity in a patient with capnocytophaga canimorsus sepsis: a case report. *Transfusion (Paris)* **58**, 2426–2429, https://doi.org/10.1111/trf.14829
62 Peetermans, M., Meyers, S., Liesenborghs, L., Vanhoorelbeke, K., De Meyer, S.F., Vandenbriele, C. et al. (2020) Von Willebrand factor and ADAMTS13 impact on the outcome of Staphylococcus aureus sepsis. *J. Thromb. Haemost.* **18**, 722–731, https://doi.org/10.1111/jth.14686
63 Czaikoski, P.G., Mota, J.M.S.C., Nascimento, D.C., Sônego, F., e Silva Castanheira, F.V., Melo, P.H. et al. (2016) Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. *PLoS ONE* **11**, e0148142, https://doi.org/10.1371/journal.pone.0148142
64 Stiel, L., Mayeur-Rousse, C., Helms, J., Meziani, F. and Mauvieux, L. (2019) First visualization of circulating neutrophil extracellular traps using cell fluorescence during human septic shock-induced disseminated intravascular coagulation. *Thromb. Res.* **183**, 153–158, https://doi.org/10.1016/j.thromres.2019.09.036
65 Mao, J.-Y., Zhang, J.-H., Cheng, W., Chen, J.-W. and Cui, N. (2021) Effects of neutrophil extracellular traps in patients with septic coagulopathy and their interaction with autophagy. *Front. Immunol.* **12**, https://doi.org/10.3389/fimmu.2021.757041
66 Colón, D.F., Wanderley, C.W., Franchin, M., Silva, C.M., Hiroki, C.H., Castanheira, F.V.S. et al. (2019) Neutrophil extracellular traps (NETs) exacerbate severity of infant sepsis. *Crit. Care* **23**, 113, https://doi.org/10.1186/s13054-019-2407-8
67 Shorr, A.F., Bernard, G.R., Dhainaut, J.-F., Russell, J.R., Macias, W.L., Nelson, D.R. et al. (2006) Protein C concentrations in severe sepsis: an early directional change in plasma levels predicts outcome. *Crit. Care* **10**, R92, https://doi.org/10.1186/cc4946
68 Bernard, G.R., Vincent, J.-L., Laterre, P.-F., LaRosa, S.P., Dhainaut, J.-F., Lopez-Rodriguez, A. et al. (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. *N. Engl. J. Med.* **344**, 699–709, https://doi.org/10.1056/NEJM200103083441001
69 Martí-Carvajal, A.J., Solà, I., Gluud, C., Lathyris, D. and Anand, V. (2012) Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. *Cochrane Database Syst. Rev.* **12**, https://doi.org/10.1002/14651858.CD004388.pub5
70 Kurosawa, S., Stearns-Kurosawa, D.J., Carson, C.W., Angelo, A.D., Della Valle, P. and Esmon, C.T. (1998) Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. *Blood* **91**, 725–727, https://doi.org/10.1182/blood.V91.2.725
71 Watanabe, E., Takasu, O., Teratake, Y., Sakamoto, T., Ikeda, T., Kotani, J. et al. (2022) A thrombomodulin promoter gene polymorphism, Rs2239562, Influences both susceptibility to and outcome of sepsis. *Front. Med.* **8**, https://doi.org/10.3389/fmed.2021.762198
72 Moxon, C.A., Alhamdi, Y., Storm, J., Toh, J.M.H., McGuinness, D., Ko, J.Y. et al. (2020) Parasite histones are toxic to brain endothelium and link blood barrier breakdown and thrombosis in cerebral malaria. *Blood Adv.* **4**, 2851–2864, https://doi.org/10.1182/bloodadvances.2019001258
73 Riedl, J., Mordmüller, B., Koder, S., Pabinger, I., Kremsner, P.G., Hoffman, S.L. et al. (2016) Alterations of blood coagulation in controlled human malaria infection. *Malar. J.* **15**, 15, https://doi.org/10.1186/s12936-015-1079-3
74 Bridges, D.J., Bunn, J., van Mourik, J.A., Grau, G., Preston, R.J.S., Molyneux, M. et al. (2010) Rapid activation of endothelial cells enables plasmodium falciparum adhesion to platelet-decorated von willebrand factor strings. *Blood* **115**, 1472–1474, https://doi.org/10.1182/blood-2009-07-235150
75 Helms, J., Tacquard, C., Severac, F., Leonard-Lorant, I., Ohana, M., Delabranche, X. et al. (2020) High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* **46**, 1089–1098, https://doi.org/10.1007/s00134-020-06062-x
76 Middeldorp, S., Coppens, M., van Haaps, T.F., Foppen, M., Vlaar, A.P., Müller, M.C.A. et al. (2020) Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J. Thromb. Haemost.* **18**, 1995–2002, https://doi.org/10.1111/jth.14888
77 Hill, J.B., Garcia, D., Crowther, M., Savage, B., Peress, S., Chang, K. et al. (2020) Frequency of venous thromboembolism in 6513 patients with COVID-19: a retrospective study. *Blood Adv.* **4**, 5373–5377, https://doi.org/10.1182/bloodadvances.2020003083
78 Piazza, G., Campia, U., Hurwitz, S., Snyder, J.E., Rizzo, S.M., Pfeferman, M.B. et al. (2020) Registry of arterial and venous thromboembolic complications in patients with COVID-19. *J. Am. Coll. Cardiol.* **76**, 2060–2072, https://doi.org/10.1016/j.jacc.2020.08.070
79 Liu, H., Hu, T., Zhang, C., Chen, X., Zhang, S., Li, M. et al. (2021) Mechanisms of COVID-19 thrombosis in an inflammatory environment and new anticoagulant targets. *Am. J. Transl. Res.* **13**, 3925–3941
80 Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J.A. et al. (2020) Neutrophil extracellular traps in COVID-19. *JCI Insight* **5**, https://doi.org/10.1172/jci.insight.138999
81 Rohlfing, A.K., Rath, D., Geisler, T. and Gawaz, M. (2021) Platelets and COVID-19. *Hamostaseologie* **41**, 379–385, https://doi.org/10.1055/a-1581-4355

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

82 Langnau, C., Rohlfing, A.K., Gekeler, S., Günter, M., Pöschel, S., Petersen-Uribe, Á. et al. (2021) Platelet activation and plasma levels of furin are associated with prognosis of patients with coronary artery disease and COVID-19. *Arter. Thromb. Vasc. Biol.* **41**, 2080–2096, https://doi.org/10.1161/ATVBAHA.120.315698

83 Borges do Nascimento, I.J., von Groote, T.C., O'Mathúna, D.P., Abdulazeem, H.M., Henderson, C., Jayarajah, U. et al. (2020) Clinical, laboratory and radiological characteristics and outcomes of novel coronavirus (SARS-CoV-2) infection in humans: a systematic review and series of meta-analyses. *PLoS ONE* **15**, e0239235, https://doi.org/10.1371/journal.pone.0239235

84 Wang, C., Yu, C., Jing, H., Wu, X., Novakovic, V.A., Xie, R. et al. (2022) long covid: The Nature of Thrombotic Sequelae Determines the Necessity of Early Anticoagulation. *Front. Cell Infect. Microbiol.* **12**, 861703, https://doi.org/10.3389/fcimb.2022.861703

85 Fogarty, H., Ward, S.E., Townsend, L., Karampini, E., Elliott, S., Conlon, N. et al. (2022) Sustained VWF-ADAMTS-13 Axis Imbalance and Endotheliopathy in Long COVID Syndrome Is Related to Immune Dysfunction. *J. Thromb. Haemost.*, jth.15830, https://doi.org/10.1111/jth.15830

86 Luther, N., Shahneh, F., Brähler, M., Krebs, F., Jäckel, S., Subramaniam, S. et al. (2016) Innate effector-memory T-cell activation regulates post-thrombotic vein wall inflammation and thrombus resolution. *Circ. Res.* **119**, 1286–1295, https://doi.org/10.1161/CIRCRESAHA.116.309301

87 Nosaka, M., Ishida, Y., Kimura, A., Kuninaka, Y., Inui, M., Mukaida, N. et al. (2011) Absence of IFN-γ accelerates thrombus resolution through enhanced MMP-9 and VEGF Expression in mice. *J. Clin. Invest.* **121**, 2911–2920, https://doi.org/10.1172/JCI40782

88 Shahneh, F., Grill, A., Klein, M., Frauhammer, F., Bopp, T., Schäfer, K. et al. (2021) Specialized regulatory T cells control venous blood clot resolution through SPARC. *Blood* **137**, 1517–1526, https://doi.org/10.1182/blood.2020005407

89 Risk of Venous and Arterial Thromboembolic Events in Patients Receiving Targeted Anti-cancer Therapy - A Nationwide Cohort Study. ISTH Congress Abstracts, https://abstracts.isth.org/abstract/risk-of-venous-and-arterial-thromboembolic-events-in-patients-receiving-targeted-anti-cancer-therapy-a-nationwide-cohort-study/ 2022-11-02)

90 Wang, T.-F., Khorana, A.A. and Carrier, M. (2021) Thrombotic complications associated with immune checkpoint inhibitors. *Cancers* **13**, 4606, https://doi.org/10.3390/cancers13184606

91 Wang, L., Wang, F.S. and Gershwin, M.E. (2015) Human Autoimmune diseases: a comprehensive update. *J. Intern. Med.* **278**, 369–395, https://doi.org/10.1111/joim.12395

92 Garcia, P. (2018) Are you autoimmune aware?, www.JDRF.org.uk Accessed: 30/08/2022

93 Ng, S.C., Shi, H.Y., Hamidi, N., Underwood, F.E., Tang, W., Benchimol, E.I. et al. (2017) Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet Lond. Engl.* **390**, 2769–2778, https://doi.org/10.1016/S0140-6736(17)32448-0

94 Rosen, M.J., Dhawan, A. and Saeed, S.A. (2015) Inflammatory bowel disease in children and adolescents. *JAMA Pediatr.* **169**, 1053–1060, https://doi.org/10.1001/jamapediatrics.2015.1982

95 Benchimol, E.I., Bernstein, C.N., Bitton, A., Carroll, M.W., Singh, H., Otley, A.R. et al. (2017) Trends in epidemiology of pediatric inflammatory bowel disease in Canada: distributed network analysis of multiple population-based provincial health administrative databases. *Am. J. Gastroenterol.* **112**, 1120–1134, https://doi.org/10.1038/ajg.2017.97

96 Naito, T., Botwin, G.J., Haritunians, T., Li, D., Yang, S., Khrom, M. et al. (2021) Prevalence and effect of genetic risk of thromboembolic disease in inflammatory bowel disease. *Gastroenterology* **160**, 771.e4–780.e4, https://doi.org/10.1053/j.gastro.2020.10.019

97 Kappelman, M.D., Horvath-Puho, E., Sandler, R.S., Rubin, D.T., Ullman, T.A., Pedersen, L. et al. (2011) Thromboembolic risk among Danish children and adults with inflammatory bowel diseases: a population-based nationwide study. *Gut* **60**, 937–943, https://doi.org/10.1136/gut.2010.228585

98 Grainge, M.J., West, J. and Card, T.R. (2010) Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet* **375**, 657–663, https://doi.org/10.1016/S0140-6736(09)61963-2

99 Lee, S.H., Kwon, J.E. and Cho, M.L. (2018) Immunological pathogenesis of inflammatory bowel disease. *Intest. Res.* **16**, 26–42, https://doi.org/10.5217/ir.2018.16.1.26

100 Medina-Contreras, O., Harusato, A., Nishio, H., Flannigan, K.L., Ngo, V., Leoni, G. et al. (2016) Cutting edge: IL-36 receptor promotes resolution of intestinal damage. *J. Immunol.* **196**, 34–38, https://doi.org/10.4049/jimmunol.1501312

101 Leon, G., Hernandez Santana, Y.E., Irwin, N., Giannoudaki, E., O'Neill, S., Csizmadia, I. et al. (2022) IL-36 cytokines imprint a colitogenic phenotype on CD4+ T helper cells. *Mucosal. Immunol.* **15**, 491–503, https://doi.org/10.1038/s41385-022-00488-w

102 Jones, G.R., Bain, C.C., Fenton, T.M., Kelly, A., Brown, S.L., Ivens, A.C. et al. (2018) Dynamics of colon monocyte and macrophage activation during colitis. *Front. Immunol.* **9**, 2764, https://doi.org/10.3389/fimmu.2018.02764

103 Alkim, H., Ayaz, S., Alkim, C., Ulker, A. and Sahin, B. (2011) Continuous active state of coagulation system in patients with nonthrombotic inflammatory bowel disease. *Clin. Appl. Thromb. Hemost.* **17**, 600–604, https://doi.org/10.1177/1076029611405034

104 Kume, K., Yamasaki, M., Tashiro, M., Yoshikawa, I. and Otsuki, M. (2007) Activations of coagulation and fibrinolysis secondary to bowel inflammation in patients with ulcerative colitis. *Intern. Med.* **46**, 1323–1329, https://doi.org/10.2169/internalmedicine.46.0237

105 Cakal, B., Gokmen, A., Yalinkilic, M., Cakal, E., Ayaz, S., Nadir, I. et al. (2010) Natural anticoagulant protein levels in Turkish patients with inflammatory bowel disease. *Blood Coagul. Fibrinolysis* **21**, 118–121, https://doi.org/10.1097/MBC.0b013e328335d025

106 Dolapcioglu, C., Soylu, A., Kendir, T., Ince, A.T., Dolapcioglu, H., Purisa, S. et al. (2014) Coagulation parameters in inflammatory bowel disease. *Int. J. Clin. Exp. Med.* **7**, 1442–1448

107 Anthoni, C., Russell, J., Wood, K.C., Stokes, K.Y., Vowinkel, T., Kirchhofer, D. et al. (2007) Tissue factor: a mediator of inflammatory cell recruitment, tissue injury, and thrombus formation in experimental colitis. *J. Exp. Med.* **204**, 1595–1601, https://doi.org/10.1084/jem.20062354

108 Palkovits, J., Novacek, G., Kollars, M., Hron, G., Osterode, W., Quehenberger, P. et al. (2013) Tissue factor exposing microparticles in inflammatory bowel disease. *J. Crohns. Colitis* **7**, 222–229, https://doi.org/10.1016/j.crohns.2012.05.016

109 Deutschmann, A., Schlagenhauf, A., Leschnik, B., Hoffmann, K.M., Hauer, A. and Muntean, W. (2013) Increased procoagulant function of microparticles in pediatric inflammatory bowel disease: role in increased thrombin generation. *J. Pediatr. Gastroenterol. Nutr.* **56**, 401–407, https://doi.org/10.1097/MPG.0b013e31827daf72
110 Moschonas, I. and Tselepis, A. (2018) Platelet-derived microparticles induce the formation of neutrophil extracellular traps. *Atherosclerosis* **275**, e106, https://doi.org/10.1016/j.atherosclerosis.2018.06.291
111 Wang, Y., Luo, L., Braun, O., Westman, J., Madhi, R., Herwald, H. et al. (2018) Neutrophil extracellular trap-microparticle complexes enhance thrombin generation via the intrinsic pathway of coagulation in mice. *Sci. Rep.* **8**, 4020, https://doi.org/10.1038/s41598-018-22156-5
112 Collins, C.E., Cahill, M.R., Newland, A.C. and Rampton, D.S. (1994) Platelets circulate in an activated state in inflammatory bowel disease. *Gastroenterology* **106**, 840–845, https://doi.org/10.1016/0016-5085(94)90741-2
113 Harries, A.D., Fitzsimons, E., Fifield, R., Dew, M.J. and Rhoades, J. (1983) Platelet count: a simple measure of activity in Crohn's disease. *Br. Med. J. (Clin. Res. Ed)* **286**, 1476, https://doi.org/10.1136/bmj.286.6376.1476
114 Vetrano, S., Ploplis, V.A., Sala, E., Sandoval-Cooper, M., Donahue, D.L., Correale, C. et al. (2011) Unexpected role of anticoagulant protein C in controlling epithelial barrier integrity and intestinal inflammation. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19830–19835, https://doi.org/10.1073/pnas.1107140108
115 Scaldaferri, F., Sans, M., Vetrano, S., Graziani, C., De Cristofaro, R., Gerlitz, B. et al. (2007) Crucial role of the protein C pathway in governing microvascular inflammation in inflammatory bowel disease. *J. Clin. Invest.* **117**, 1951–1960, https://doi.org/10.1172/JCI31027
116 Lay, A.J., Liang, Z., Rosen, E.D. and Castellino, F.J. (2005) Mice with a severe deficiency in protein C display prothrombotic and proinflammatory phenotypes and compromised maternal reproductive capabilities. *J. Clin. Invest.* **115**, 1552–1561, https://doi.org/10.1172/JCI24030
117 Kondreddy, V., Keshava, S., Esmon, C.T., Pendurthi, U.R. and Rao, L.V.M. (2020) A critical role of endothelial cell protein C receptor in the intestinal homeostasis in experimental colitis. *Sci. Rep.* **10**, 20569, https://doi.org/10.1038/s41598-020-77502-3
118 Sarlos, P., Szemes, K., Hegyi, P., Garami, A., Szabo, I., Illes, A. et al. (2018) Steroid but not biological therapy elevates the risk of venous thromboembolic events in inflammatory bowel disease: a meta-analysis. *J. Crohns. Colitis* **12**, 489–498, https://doi.org/10.1093/ecco-jcc/jjx162
119 Poizeau, F., Nowak, E., Kerbrat, S., Le Nautout, B., Droitcourt, C., Drici, M.-D. et al. (2020) Association between early severe cardiovascular events and the initiation of treatment with the anti-interleukin 12/23p40 antibody ustekinumab. *JAMA Dermatol.* **156**, 1208–1215, https://doi.org/10.1001/jamadermatol.2020.2977
120 Levine, I., Rolston, V., Papademetriou, M. and Malter, L. (2018) Ustekinumab-associated thrombocytopenia in a patient with refractory Crohn's disease: 2044. *Off. J. Am. Coll. Gastroenterol. ACG* **113**, S1166, https://doi.org/10.14309/00000434-201810001-02044
121 Detrez, I., Ballet, V., Peeters, M., Van Assche, G., Vermeire, S., Ferrante, M. et al. (2018) P484 infliximab and vedolizumab show a different effect on clot formation in inflammatory bowel disease patients. *J. Crohns Colitis* **12**, S349–S350, https://doi.org/10.1093/ecco-jcc/jjx180.611
122 Kekre, N., Kim, H.T., Ho, V.T., Cutler, C., Armand, P., Nikiforow, S. et al. (2017) Venous thromboembolism is associated with graft-. *Haematologica* **102**, 1185–1191, https://doi.org/10.3324/haematol.2017.164012
123 de Lima, M., Anagnostopoulos, A., Munsell, M., Shahjahan, M., Ueno, N., Ippoliti, C. et al. (2004) Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood* **104**, 865–872, https://doi.org/10.1182/blood-2003-11-3750
124 Russell, J.A., Duan, Q., Chaudhry, M.A., Savoie, M.L., Balogh, A., Turner, A.R. et al. (2008) Transplantation from matched siblings using once-daily intravenous busulfan/fludarabine with thymoglobulin: a myeloablative regimen with low nonrelapse mortality in all but older patients with high-risk disease. *Biol. Blood Marrow Transpl.* **14**, 888–895, https://doi.org/10.1016/j.bbmt.2008.05.010
125 Maris, M.B., Sandmaier, B.M., Storer, B.E., Maloney, D.G., Shizuru, J.A., Agura, E. et al. (2006) Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. *Biol. Blood Marrow Transpl.* **12**, 454–465, https://doi.org/10.1016/j.bbmt.2005.12.030
126 Gonsalves, A., Carrier, M., Wells, P.S., McDiarmid, S.A., Huebsch, L.B. and Allan, D.S. (2008) Incidence of symptomatic venous thromboembolism following hematopoietic stem cell transplantation. *J. Thromb. Haemost.* **6**, 1468–1473, https://doi.org/10.1111/j.1538-7836.2008.03077.x
127 Azık, F., Gökçebay, D.G., Tavil, B., Işık, P., Tunç, B. and Uçkan, D. (2015) Venous thromboembolism after allogeneic pediatric hematopoietic stem cell transplantation: a single-center study. *Turk J. Haematol.* **32**, 228–233, https://doi.org/10.4274/tjh.2013.0066
128 Labrador, J., González-Rivero, J., Monroy, R., Lozano, F.S., López-Corral, L., Caballero, M.D. et al. (2016) Management patterns and outcomes in symptomatic venous thromboembolism following allogeneic hematopoietic stem cell transplantation. A 15-year experience at a single center. *Thromb. Res.* **142**, 52–56, https://doi.org/10.1016/j.thromres.2016.02.016
129 Gerber, D.E., Segal, J.B., Levy, M.Y., Kane, J., Jones, R.J. and Streiff, M.B. (2008) The incidence of and risk factors for venous thromboembolism (VTE) and bleeding among 1514 patients undergoing hematopoietic stem cell transplantation: implications for VTE prevention. *Blood* **112**, 504–510, https://doi.org/10.1182/blood-2007-10-117051
130 El Jurdi, N., Elhusseini, H., Beckman, J., DeFor, T.E., Okoev, G., Rogosheske, J. et al. (2021) High incidence of thromboembolism in patients with chronic GVHD: association with severity of GVHD and donor-recipient ABO blood group. *Blood Cancer J.* **11**, 96, https://doi.org/10.1038/s41408-021-00488-2
131 Laskin, B.L., Goebel, J., Davies, S.M. and Jodele, S. (2011) Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Blood* **118**, 1452–1462, https://doi.org/10.1182/blood-2011-02-321315
132 Gloude, N.J., Khandelwal, P., Luebbering, N., Lounder, D.T., Jodele, S., Alder, M.N. et al. (2017) Circulating DsDNA, endothelial injury, and complement activation in thrombotic microangiopathy and GVHD. *Blood* **130**, 1259–1266, https://doi.org/10.1182/blood-2017-05-782870
133 Amoroso, A., Mitterhofer, A.P., Del Porto, F., Garzia, P., Ferri, G.M., Galluzzo, S. et al. (2003) Antibodies to anionic phospholipids and anti-beta2-GPI: association with thrombosis and thrombocytopenia in systemic lupus erythematosus. *Hum. Immunol.* **64**, 265–273, https://doi.org/10.1016/S0198-8859(02)00789-9

134 Love, P.E. and Santoro, S.A. (1990) Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. prevalence and clinical significance. *Ann. Intern. Med.* **112**, 682–698, https://doi.org/10.7326/0003-4819-112-9-682

135 Gustafsson, J., Gunnarsson, I., Börjesson, O., Pettersson, S., Möller, S., Fei, G.Z. et al. (2009) Predictors of the first cardiovascular event in patients with systemic lupus erythematosus - a prospective cohort study. *Arthritis Res. Ther.* **11**, R186, https://doi.org/10.1186/ar2878

136 Noordermeer, T., Molhoek, J.E., Schutgens, R.E.G., Sebastian, S.A.E., Drost-Verhoef, S., van Wesel, A.C.W. et al. (2021) Anti-β2-glycoprotein I and anti-prothrombin antibodies cause lupus anticoagulant through different mechanisms of action. *J. Thromb. Haemost.* **19**, 1018–1028, https://doi.org/10.1111/jth.15241

137 Ohl, K. and Tenbrock, K. (2011) Inflammatory cytokines in systemic lupus erythematosus. *J. Biomed. Biotechnol.* **2011**, 432595, https://doi.org/10.1155/2011/432595

138 Rauch, J., Salem, D., Subang, R., Kuwana, M. and Levine, J.S. (2018) β2-glycoprotein I-reactive T cells in autoimmune disease. *Front. Immunol.* **9**, 2836, https://doi.org/10.3389/fimmu.2018.02836

139 Müller-Calleja, N., Hollerbach, A., Royce, J., Ritter, S., Pedrosa, D., Madhusudhan, T. et al. (2021) Lipid presentation by the protein c receptor links coagulation with autoimmunity. *Science* **371**, https://doi.org/10.1126/science.abc0956

140 Takahagi, S., Mihara, S., Iwamoto, K., Morioke, S., Okabe, T., Kameyoshi, Y. et al. (2010) Coagulation/fibrinolysis and inflammation markers are associated with disease activity in patients with chronic urticaria. *Allergy* **65**, 649–656, https://doi.org/10.1111/j.1398-9995.2009.02222.x

141 Yanase, Y., Takahagi, S. and Hide, M. (2018) Chronic spontaneous urticaria and the extrinsic coagulation system. *Allergol. Int.* **67**, 191–194, https://doi.org/10.1016/j.alit.2017.09.003

142 Xue, M., Lin, H., Zhao, R., Fryer, C., March, L. and Jackson, C.J. (2022) Activated protein C protects against murine contact dermatitis by suppressing protease-activated receptor 2. *Int. J. Mol. Sci.* **23**, https://doi.org/10.3390/ijms23010516

143 Brims, F.J., Chauhan, A.J., Higgins, B. and Shute, J.K. (2009) Coagulation factors in the airways in moderate and severe asthma and the effect of inhaled steroids. *Thorax* **64**, 1037–1043, https://doi.org/10.1136/thx.2009.114439

144 Chung, W.S., Lin, C.L., Ho, F.M., Li, R.Y., Sung, F.C., Kao, C.H. et al. (2014) Asthma increases pulmonary thromboembolism risk: a nationwide population cohort study. *Eur. Respir. J.* **43**, 801–807, https://doi.org/10.1183/09031936.00043313

145 Mitchel, J.A., Antoniak, S., Lee, J.H., Kim, S.H., McGill, M., Kasahara, D.I. et al. (2016) IL-13 augments compressive stress-induced tissue factor expression in human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **54**, 524–531, https://doi.org/10.1165/rcmb.2015-0252OC

146 Shinagawa, K., Martin, J.A., Ploplis, V.A. and Castellino, F.J. (2007) Coagulation factor Xa modulates airway remodeling in a murine model of asthma. *Am. J. Respir. Crit. Care Med.* **175**, 136–143, https://doi.org/10.1164/rccm.200608-1097OC

147 Blanc-Brude, O.P., Archer, F., Leoni, P., Derian, C., Bolsover, S., Laurent, G.J. et al. (2005) Factor Xa Stimulates fibroblast procollagen production, proliferation, and calcium signaling via PAR1 activation. *Exp. Cell. Res.* **304**, 16–27, https://doi.org/10.1016/j.yexcr.2004.10.021

148 Reed, C.E. and Kita, H. (2004) The role of protease activation of inflammation in allergic respiratory diseases. *J. Allergy Clin. Immunol.* **114**, 997–1008, quiz 1009, https://doi.org/10.1016/j.jaci.2004.07.060

149 Schouten, M., van de Pol, M.A., Levi, M., van der Poll, T. and van der Zee, J.S. (2009) Early activation of coagulation after allergen challenge in patients with allergic asthma. *J. Thromb. Haemost.* **7**, 1592–1594, https://doi.org/10.1111/j.1538-7836.2009.03523.x

150 Hataji, O., Taguchi, O., Gabazza, E.C., Yuda, H., Fujimoto, H., Suzuki, K. et al. (2002) Activation of protein C pathway in the airways. *Lung* **180**, 47–59, https://doi.org/10.1007/s004080000080

151 Kornerup, K.N. and Page, C.P. (2007) The role of platelets in the pathophysiology of asthma. *Platelets* **18**, 319–328, https://doi.org/10.1080/09537100701230436

152 Jennewein, C., Tran, N., Paulus, P., Ellinghaus, P., Eble, J.A. and Zacharowski, K. (2011) Novel aspects of fibrin(ogen) fragments during inflammation. *Mol. Med.* **17**, 568–573, https://doi.org/10.2119/molmed.2010.00146

153 Levi, M., Schultz, M.J., Rijneveld, A.W. and van der Poll, T. (2003) Bronchoalveolar coagulation and fibrinolysis in endotoxemia and pneumonia. *Crit. Care Med.* **31**, S238–S242, https://doi.org/10.1097/01.CCM.0000057849.53689.65

154 Robson, S.C., Shephard, E.G. and Kirsch, R.E. (1994) Fibrin degradation product D-dimer induces the synthesis and release of biologically active IL-1 beta, IL-6 and plasminogen activator inhibitors from monocytes in vitro. *Br. J. Haematol.* **86**, 322–326, https://doi.org/10.1111/j.1365-2141.1994.tb04733.x

155 Lloyd-Lavery, A., Solman, L., Grindlay, D.J.C., Rogers, N.K., Thomas, K.S. and Harman, K.E. (2019) What's new in atopic eczema? An analysis of systematic reviews published in 2016. Part 2: epidemiology, aetiology and risk factors. *Clin. Exp. Dermatol.* **44**, 370–375, https://doi.org/10.1111/ced.13853

156 Nassau, S. and Fonacier, L. (2020) Allergic contact dermatitis. *Med. Clin. North Am.* **104**, 61–76, https://doi.org/10.1016/j.mcna.2019.08.012

157 Gittler, J.K., Shemer, A., Suárez-Fariñas, M., Fuentes-Duculan, J., Gulewicz, K.J., Wang, C.Q. et al. (2012) Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J. Allergy Clin. Immunol.* **130**, 1344–1354, https://doi.org/10.1016/j.jaci.2012.07.012

158 Saito, R., Yanase, Y., Kamegashira, A., Takahagi, S., Tanaka, A., Uchida, K. et al. (2020) Increase of tissue factor expression on the surface of peripheral monocytes of patients with chronic spontaneous urticaria. *Allergy* **75**, 971–974, https://doi.org/10.1111/all.14110

159 Yanase, Y., Matsuo, Y., Takahagi, S., Kawaguchi, T., Uchida, K., Ishii, K. et al. (2021) Coagulation factors induce human skin mast cell and basophil degranulation via activation of complement 5 and the C5a receptor. *J. Allergy Clin. Immunol.* **147**, 1101.e7–1104.e7, https://doi.org/10.1016/j.jaci.2020.08.018

160 Sherenian, M., Filuta, A., Amezcua, P., Ruff, B., Kroner, J., Grashel, B. et al. (2022) Thrombin and fibrinogen play a critical role in atopic dermatitis pathogenesis. *J. Allergy Clin. Immunol.* **149**, AB6, https://doi.org/10.1016/j.jaci.2021.12.060

161 Yadav, S.K., Mindur, J.E., Ito, K. and Dhib-Jalbut, S. (2015) Advances in the immunopathogenesis of multiple sclerosis. *Curr. Opin. Neurol.* **28**, 206–219, https://doi.org/10.1097/WCO.0000000000000205

162 Ahmed, O., Geraldes, R., DeLuca, G.C. and Palace, J. (2019) Multiple sclerosis and the risk of systemic venous thrombosis: a systematic review. *Mult. Scler Relat. Disord.* **27**, 424–430, https://doi.org/10.1016/j.msard.2018.10.008
163 Putnam, T.J. (1937) Lesions of "encephalomyelitis" and multiple sclerosis: venous thrombosis as the primary alteration. *J. Am. Med. Assoc.* **108**, 1477–1480, https://doi.org/10.1001/jama.1937.02780180001001
164 Putnam, T. (1936) Studies in multiple sclerosis. *Ann. Intern. Med.* **9**, 854–863, https://doi.org/10.7326/0003-4819-9-7-854
165 Han, M.H., Hwang, S.I., Roy, D.B., Lundgren, D.H., Price, J.V., Ousman, S.S. et al. (2008) Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature* **451**, 1076–1081, https://doi.org/10.1038/nature06559
166 Cuzner, M.L., Gveric, D., Strand, C., Loughlin, A.J., Paemen, L., Opdenakker, G. et al. (1996) The expression of tissue-type plasminogen activator, matrix metalloproteases and endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. *J. Neuropathol. Exp. Neurol.* **55**, 1194–1204, https://doi.org/10.1097/00005072-199612000-00002
167 Gveric, D., Herrera, B., Petzold, A., Lawrence, D.A. and Cuzner, M.L. (2003) Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. *Brain* **126**, 1590–1598, https://doi.org/10.1093/brain/awg167
168 Kohriyama, T., Maruyama, H., Kurokawa, K., Harada, T. and Nakamura, S. (1997) Endothelial cell activation and/or injury in multiple sclerosis: analysis with von Willebrand factor and thrombomodulin. *Rinsho Shinkeigaku* **37**, 287–291
169 Tsukada, N., Matsuda, M., Miyagi, K. and Yanagisawa, N. (1995) Thrombomodulin in the sera of patients with multiple sclerosis and human lymphotropic virus type-1-associated myelopathy. *J. Neuroimmunol.* **56**, 113–116, https://doi.org/10.1016/0165-5728(94)00156-I
170 Ryu, J.K., Rafalski, V.A., Meyer-Franke, A., Adams, R.A., Poda, S.B., Rios Coronado, P.E. et al. (2018) Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration. *Nat. Immunol.* **19**, 1212–1223, https://doi.org/10.1038/s41590-018-0232-x
171 Göbel, K., Pankratz, S., Asaridou, C.M., Herrmann, A.M., Bittner, S., Merker, M. et al. (2016) Blood coagulation factor XII drives adaptive immunity during neuroinflammation via CD87-Mediated Modulation of Dendritic Cells. *Nat Commun* **7**, 11626, https://doi.org/10.1038/ncomms11626
172 Ziliotto, N., Baroni, M., Straudi, S., Manfredini, F., Mari, R., Menegatti, E. et al. (2018) Coagulation factor XII levels and intrinsic thrombin generation in multiple sclerosis. *Front. Neurol.* **9**, 245, https://doi.org/10.3389/fneur.2018.00245
173 Chan, N.C. and Weitz, J.I. (2019) Antithrombotic agents. *Circ. Res.* **124**, 426–436, https://doi.org/10.1161/CIRCRESAHA.118.313155
174 Bickmann, J.K., Baglin, T., Meijers, J.C.M. and Renné, T. (2017) Novel targets for anticoagulants lacking bleeding risk. *Curr. Opin. Hematol.* **24**, 419–426, https://doi.org/10.1097/MOH.0000000000000367
175 Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. and Santoro, A. (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* **14**, 576–590, https://doi.org/10.1038/s41574-018-0059-4
176 Price, J., Lord, J.M. and Harrison, P. (2020) Inflammaging and platelet hyperreactivity: a new therapeutic target? *J. Thromb. Haemost.* **18**, 3–5, https://doi.org/10.1111/jth.14670
177 Culmer, D.L., Diaz, J.A., Hawley, A.E., Jackson, T.O., Shuster, K.A., Sigler, R.E. et al. (2013) Circulating and vein wall P-selectin promote venous thrombogenesis during aging in a rodent model. *Thromb. Res.* **131**, 42–48, https://doi.org/10.1016/j.thromres.2012.10.013
178 Dayal, S., Wilson, K.M., Motto, D.G., Miller, F.J., Chauhan, A.K. and Lentz, S.R. (2013) Hydrogen peroxide promotes aging-related platelet hyperactivation and thrombosis. *Circulation* **127**, 1308–1316, https://doi.org/10.1161/CIRCULATIONAHA.112.000966
179 Puchta, A., Naidoo, A., Verschoor, C.P., Loukov, D., Thevaranjan, N., Mandur, T.S. et al. (2016) TNF drives monocyte dysfunction with age and results in impaired anti-pneumococcal immunity. *PLoS Pathog.* **12**, e1005368, https://doi.org/10.1371/journal.ppat.1005368
180 Parameswaran, N. and Patial, S. (2010) Tumor necrosis factor-α signaling in macrophages. *Crit. Rev. Eukaryot. Gene Expr.* **20**, 87–103, https://doi.org/10.1615/CritRevEukarGeneExpr.v20.i2.10
181 Taylor, D.D., Senhauser, D.A. and Cavazos, F. (1968) Thrombocytopathy associated with nonleukemic megakaryocytic myelosis. functional and fine structure observations of the abnormal platelets. *Am. J. Clin. Pathol.* **49**, 662–670, https://doi.org/10.1093/ajcp/49.5.662
182 Hattori, A., Koike, K., Ito, S. and Matsuoka, M. (1975) Static and functional morphology of the pathological platelets in primary myelofibrosis and myeloproliferative syndrome. *Ser. Haematol.* **8**, 126–150
183 Stephen, J., Emerson, B., Fox, K.A. and Dransfield, I. (2013) The uncoupling of monocyte-platelet interactions from the induction of proinflammatory signaling in monocytes. *J. Immunol.* **191**, 5677–5683, https://doi.org/10.4049/jimmunol.1301250
184 van der Zee, P.M., Biró, E., Ko, Y., de Winter, R.J., Hack, C.E., Sturk, A. et al. (2006) P-selectin- and CD63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction. *Clin. Chem.* **52**, 657–664, https://doi.org/10.1373/clinchem.2005.057414
185 Iannucci, J., Renehan, W. and Grammas, P. (2020) Thrombin, a mediator of coagulation, inflammation, and neurotoxicity at the neurovascular interface: implications for Alzheimer's disease. *Front. Neurosci.* **14**, 762, https://doi.org/10.3389/fnins.2020.00762
186 Li, D.Q., Zhou, Y.P. and Yang, H. (2012) Donepezil combined with natural hirudin improves the clinical symptoms of patients with mild-to-moderate Alzheimer's disease: a 20-week open-label pilot study. *Int. J. Med. Sci.* **9**, 248–255, https://doi.org/10.7150/ijms.4363
187 Smyth, L.C.D., Murray, H.C., Hill, M., van Leeuwen, E., Highet, B., Magon, N.J. et al. (2022) Neutrophil-vascular interactions drive myeloperoxidase accumulation in the brain in Alzheimer's disease. *Acta. Neuropathol. Commun.* **10**, 38, https://doi.org/10.1186/s40478-022-01347-2
188 Yasuhara, O., Walker, D.G. and McGeer, P.L. (1994) Hageman factor and its binding sites are present in senile plaques of Alzheimer's disease. *Brain Res.* **654**, 234–240, https://doi.org/10.1016/0006-8993(94)90484-7
189 Ashby, E.L., Love, S. and Kehoe, P.G. (2012) Assessment of activation of the plasma kallikrein-kinin system in frontal and temporal cortex in Alzheimer's disease and vascular dementia. *Neurobiol. Aging* **33**, 1345–1355, https://doi.org/10.1016/j.neurobiolaging.2010.09.024
190 Bergamaschini, L., Parnetti, L., Pareyson, D., Canziani, S., Cugno, M. and Agostoni, A. (1998) Activation of the contact system in cerebrospinal fluid of patients with Alzheimer's disease. *Alzheimer Assoc. Disord.* **12**, 102–108, https://doi.org/10.1097/00002093-199806000-00008
191 Shibayama, Y., Joseph, K., Nakazawa, Y., Ghebreihiwet, B., Peerschke, E.I. and Kaplan, A.P. (1999) Zinc-dependent activation of the plasma kinin-forming cascade by aggregated beta amyloid protein. *Clin. Immunol.* **90**, 89–99, https://doi.org/10.1006/clim.1998.4621

Clinical Science (2022) **136** 1809–1829
https://doi.org/10.1042/CS20220208

192 Maas, C., Govers-Riemslag, J.W., Bouma, B., Schiks, B., Hazenberg, B.P., Lokhorst, H.M. et al. (2008) Misfolded proteins activate factor XII in humans, leading to kallikrein formation without initiating coagulation. *J. Clin. Invest.* **118**, 3208–3218, https://doi.org/10.1172/JCI35424
193 Bergamaschini, L., Donarini, C., Foddi, C., Gobbo, G., Parnetti, L. and Agostoni, A. (2001) The region 1-11 of alzheimer amyloid-beta is critical for activation of contact-kinin system. *Neurobiol. Aging* **22**, 63–69, https://doi.org/10.1016/S0197-4580(00)00174-3
194 Zamolodchikov, D., Chen, Z.L., Conti, B.A., Renné, T. and Strickland, S. (2015) Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 4068–4073, https://doi.org/10.1073/pnas.1423764112
195 Chen, Z.L., Revenko, A.S., Singh, P., MacLeod, A.R., Norris, E.H. and Strickland, S. (2017) Depletion of coagulation factor XII ameliorates brain pathology and cognitive impairment in Alzheimer's disease mice. *Blood* **129**, 2547–2556, https://doi.org/10.1182/blood-2016-11-753202
196 Bisht, K., Sharma, K. and Tremblay, M.-È. (2018) Chronic stress as a risk factor for Alzheimer's disease: roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress. *Neurobiol. Stress* **9**, 9–21, https://doi.org/10.1016/j.ynstr.2018.05.003
197 Song, H., Sieurin, J., Wirdefeldt, K., Pedersen, N.L., Almqvist, C., Larsson, H. et al. (2020) Association of stress-related disorders with subsequent neurodegenerative diseases. *JAMA Neurol.* **77**, 700–709, https://doi.org/10.1001/jamaneurol.2020.0117
198 Chandola, T., Brunner, E. and Marmot, M. (2006) Chronic stress at work and the metabolic syndrome: prospective study. *BMJ* **332**, 521–525, https://doi.org/10.1136/bmj.38693.435301.80
199 Dai, S., Mo, Y., Wang, Y., Xiang, B., Liao, Q., Zhou, M. et al. (2020) Chronic stress promotes cancer development. *Front. Oncol.* **10**, 1492, https://doi.org/10.3389/fonc.2020.01492
200 Satyjeet, F., Naz, S., Kumar, V., Aung, N.H., Bansari, K., Irfan, S. et al. (2022) Psychological stress as a risk factor for cardiovascular disease: a case-control study. *Cureus* **12**, e10757
201 Dhabhar, F.S. and Mcewen, B.S. (1997) Acute stress enhances while chronic stress suppresses cell-mediated immunityin vivo: a potential role for leukocyte trafficking. *Brain Behav. Immun.* **11**, 286–306, https://doi.org/10.1006/brbi.1997.0508
202 Dhabhar, F.S., Malarkey, W.B., Neri, E. and McEwen, B.S. (2012) Stress-induced redistribution of immune cells—from barracks to boulevards to battlefields: a tale of three hormones - curt richter award winner. *Psychoneuroendocrinology* **37**, 1345–1368, https://doi.org/10.1016/j.psyneuen.2012.05.008
203 von Känel, R., Merz, F., Pfister, H., Brückl, T., Zimmermann, P., Uhr, M. et al. (2020) Acute stress-induced coagulation activation in patients with remitted major depression versus healthy controls and the role of stress-specific coping. *Ann. Behav. Med.* **54**, 611–618, https://doi.org/10.1093/abm/kaaa001
204 von Känel, R., Bellingrath, S. and Kudielka, B.M. (2009) Association of vital exhaustion and depressive symptoms with changes in fibrin D-dimer to acute psychosocial stress. *J. Psychosom. Res.* **67**, 93–101, https://doi.org/10.1016/j.jpsychores.2008.12.009
205 Zgraggen, L., Fischer, J.E., Mischler, K., Preckel, D., Kudielka, B.M. and von Känel, R. (2005) Relationship between hemoconcentration and blood coagulation responses to acute mental stress. *Thromb. Res.* **115**, 175–183, https://doi.org/10.1016/j.thromres.2004.08.022
206 von Känel, R., Mills, P.J., Fainman, C. and Dimsdale, J.E. (2001) Effects of psychological stress and psychiatric disorders on blood coagulation and fibrinolysis: a biobehavioral pathway to coronary artery disease? *Psychosom. Med.* **63**, 531–544, https://doi.org/10.1097/00006842-200107000-00003
207 Eurofound (2022) Living and Working in Europe 2021. *Publ. Off. Eur. Union Luxemb.*
208 Brown, D.L., Feskanich, D., Sánchez, B.N., Rexrode, K.M., Schernhammer, E.S. and Lisabeth, L.D. (2009) Rotating night shift work and the risk of ischemic stroke. *Am. J. Epidemiol.* **169**, 1370–1377, https://doi.org/10.1093/aje/kwp056
209 Casetta, I., Granieri, E., Fallica, E., la Cecilia, O., Paolino, E. and Manfredini, R. (2002) Patient demographic and clinical features and circadian variation in onset of ischemic stroke. *Arch. Neurol.* **59**, 48–53, https://doi.org/10.1001/archneur.59.1.48
210 Early, J.O. and Curtis, A.M. (2016) Immunometabolism: is it under the eye of the clock? *Semin. Immunol.* **28**, 478–490, https://doi.org/10.1016/j.smim.2016.10.006
211 Kondratov, R.V., Kondratova, A.A., Gorbacheva, V.Y., Vykhovanets, O.V. and Antoch, M.P. (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. *Genes Dev.* **20**, 1868–1873, https://doi.org/10.1101/gad.1432206
212 Wang, J., Yin, L. and Lazar, M.A. (2006) The Orphan nuclear receptor rev-erbα regulates circadian expression of plasminogen activator inhibitor type 1*. *J. Biol. Chem.* **281**, 33842–33848, https://doi.org/10.1074/jbc.M607873200
213 Hemmeryckx, B., Frederix, L. and Lijnen, H.R. (2019) Deficiency of Bmal1 disrupts the diurnal rhythm of haemostasis. *Exp. Gerontol.* **118**, 1–8, https://doi.org/10.1016/j.exger.2018.12.017
214 Chen, L., Li, S., Nie, J., Zhao, J., Yu, S., Li, Y. et al. (2020) Bmal1 regulates coagulation factor biosynthesis in mouse liver in Streptococcus oralis infection. *Front. Cell. Infect. Microbiol.* **10**, 530190, https://doi.org/10.3389/fcimb.2020.530190
215 Takeda, N., Maemura, K., Horie, S., Oishi, K., Imai, Y., Harada, T. et al. (2007) Thrombomodulin is a clock-controlled gene in vascular endothelial cells*. *J. Biol. Chem.* **282**, 32561–32567, https://doi.org/10.1074/jbc.M705692200
216 Somanath, P.R., Podrez, E.A., Chen, J., Ma, Y., Marchant, K., Antoch, M. et al. (2011) Deficiency in core circadian protein Bmal1 is associated with a prothrombotic and vascular phenotype. *J. Cell. Physiol.* **226**, 132–140, https://doi.org/10.1002/jcp.22314
217 Budkowska, M., Lebiecka, A., Marcinowska, Z., Woźniak, J., Jastrzębska, M. and Dołęgowska, B. (2019) The circadian rhythm of selected parameters of the hemostasis system in healthy people. *Thromb. Res.* **182**, 79–88, https://doi.org/10.1016/j.thromres.2019.08.015
218 Scheer, F.A.J.L. and Shea, S.A. (2014) Human Circadian system causes a morning peak in prothrombotic plasminogen activator inhibitor-1 (PAI-1) independent of the sleep/wake cycle. *Blood* **123**, 590–593, https://doi.org/10.1182/blood-2013-07-517060
219 Adrover, J.M., Aroca-Crevillén, A., Crainiciuc, G., Ostos, F., Rojas-Vega, Y., Rubio-Ponce, A. et al. (2020) Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation. *Nat. Immunol.* **21**, 135–144, https://doi.org/10.1038/s41590-019-0571-2
220 Shahneh, F., Christian Probst, H., Wiesmann, S.C., A-Gonzalez, N., Ruf, W., Steinbrink, K. et al. (2022) Inflammatory monocyte counts determine venous blood clot formation and resolution. *Arterioscler. Thromb. Vasc. Biol.* **42**, 145–155, https://doi.org/10.1161/ATVBAHA.121.317176

221 Nguyen, K.D., Fentress, S.J., Qiu, Y., Yun, K., Cox, J.S. and Chawla, A. (2013) Circadian gene Bmal1 regulates diurnal oscillations of Ly6Chi inflammatory monocytes. *Science* **341**, 1483–1488, https://doi.org/10.1126/science.1240636
222 Scheer, F.A.J.L., Michelson, A.D., Iii, A.L.F., Evoniuk, H., Kelly, E.E., McCarthy, M. et al. (2011) The human endogenous circadian system causes greatest platelet activation during the biological morning independent of behaviors. *PLoS ONE* **6**, e24549, https://doi.org/10.1371/journal.pone.0024549
223 Fournier, S., Guenat, F., Fournier, A., Alberio, L., Bonny, O., Bertaggia Calderara, D. et al. (2018) Circadian variation of ticagrelor-induced platelet inhibition in healthy adulty. *Eur. Heart J. - Cardiovasc. Pharmacother.* **4**, 166–171, https://doi.org/10.1093/ehjcvp/pvy003
224 Shi, J., Tong, R., Zhou, M., Gao, Y., Zhao, Y., Chen, Y. et al. (2022) Circadian nuclear receptor rev-Erbα Is expressed by platelets and potentiates platelet activation and thrombus formation. *Eur. Heart J.* ehac109, https://doi.org/10.1093/eurheartj/ehac109
225 Valnegri, P., Khelfaoui, M., Dorseuil, O., Bassani, S., Lagneaux, C., Gianfelice, A. et al. (2011) A circadian clock in hippocampus is regulated by interaction between oligophrenin-1 and Rev-Erbα. *Nat. Neurosci.* **14**, 1293–1301, https://doi.org/10.1038/nn.2911
226 Khelfaoui, M., Pavlowsky, A., Powell, A.D., Valnegri, P., Cheong, K.W., Blandin, Y. et al. (2009) Inhibition of RhoA pathway rescues the endocytosis defects in oligophrenin1 mouse model of mental retardation. *Hum. Mol. Genet.* **18**, 2575–2583, https://doi.org/10.1093/hmg/ddp189
227 Figueiro, M.G., Goo, Y., Hogan, R., Plitnick, B., Lee, J., Jahangir, K. et al. (2021) Light-dark patterns mirroring shift work accelerate atherosclerosis and promote vulnerable lesion phenotypes. *J. Am. Heart Assoc.* **10**, e018151, https://doi.org/10.1161/JAHA.120.018151
228 Wang, Y., Kisler, K., Nikolakopoulou, A.M., Fernandez, J.A., Griffin, J.H. and Zlokovic, B.V. (2022) 3K3A-activated protein C protects the blood-brain barrier and neurons from accelerated ischemic injury caused by pericyte deficiency in mice. *Front. Neurosci.* **16**, 841916, https://doi.org/10.3389/fnins.2022.841916
229 Yuda, H., Adachi, Y., Taguchi, O., Gabazza, E.C., Hataji, O., Fujimoto, H. et al. (2004) Activated protein C inhibits bronchial hyperresponsiveness and Th2 cytokine expression in mice. *Blood* **103**, 2196–2204, https://doi.org/10.1182/blood-2003-06-1980
230 Sinha, R.K., Flynn, R., Zaiken, M., Paz, K., Gavin, A.L., Nemazee, D. et al. (2019) Activated protein C ameliorates chronic graft-versus-host disease by PAR1-dependent biased cell signaling on T cells. *Blood* **134**, 776–781, https://doi.org/10.1182/blood.2019001259
231 Healy, L.D., Puy, C., Fernández, J.A., Mitrugno, A., Keshari, R.S., Taku, N.A. et al. (2017) Activated protein C inhibits neutrophil extracellular trap formation. *J. Biol. Chem.* **292**, 8616–8629, https://doi.org/10.1074/jbc.M116.768309
232 de Boer, J.D., Berger, M., Majoor, C.J., Kager, L.M., Meijers, J.C., Terpstra, S. et al. (2015) Activated protein C inhibits neutrophil migration in allergic asthma: a randomised trial. *Eur. Respir. J.* **46**, 1636–1644, https://doi.org/10.1183/13993003.00459-2015
233 Ranjan, S., Goihl, A., Kohli, S., Gadi, I., Pierau, M., Shahzad, K. et al. (2017) Activated protein C protects from GvHD via PAR2/PAR3 signalling in regulatory T-cells. *Nat. Commun.* **8**, 311, https://doi.org/10.1038/s41467-017-00169-4
234 Xue, M., Dervish, S., McKelvey, K.J., March, L., Wang, F., Little, C.B. et al. (2019) Activated protein C targets immune cells and rheumatoid synovial fibroblasts to prevent inflammatory arthritis in mice. *Rheumatology* **58**, 1850–1860, https://doi.org/10.1093/rheumatology/key429
235 Riewald, M., Petrovan, R.J., Donner, A. and Ruf, W. (2003) Activated protein C signals through the thrombin receptor PAR1 in endothelial cells. *J. Endotoxin Res.* **9**, 317–321, https://doi.org/10.1177/09680519030090050801
236 Riewald, M., Petrovan, R.J., Donner, A., Mueller, B.M. and Ruf, W. (2002) Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* **296**, 1880–1882, https://doi.org/10.1126/science.1071699
237 Lyden, P., Levy, H., Weymer, S., Pryor, K., Kramer, W., Griffin, J. et al. (2014) Phase 1 safety, tolerability and pharmacokinetics of 3K3A-APC in healthy adult volunteers. *Curr. Pharm. Des.* **19**, 7479–7485, https://doi.org/10.2174/13816128196661230131454
238 Yuda, H., Adachi, Y., Taguchi, O., Gabazza, E.C., Hataji, O., Fujimoto, H. et al. (2004) Activated protein C inhibits bronchial hyperresponsiveness and Th2 cytokine expression in mice. *Blood* **103**, 2196–2204, https://doi.org/10.1182/blood-2003-06-1980
239 Matsumoto, T., Matsushima, Y., Toda, M., Roeen, Z., D'Alessandro-Gabazza, C.N., Hinneh, J.A. et al. (2015) Activated protein C modulates the proinflammatory activity of dendritic cells. *J. Asthma Allergy* **8**, 29–37
240 Mackman, N. (2004) Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1015–1022, https://doi.org/10.1161/01.ATV.0000130465.23430.74
241 Peyvandi, F., Palla, R., Menegatti, M., Siboni, S.M., Halimeh, S., Faeser, B. et al. (2012) Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the european network of rare bleeding disorders. *J. Thromb. Haemost.* **10**, 615–621, https://doi.org/10.1111/j.1538-7836.2012.04653.x
242 Carmeliet, P., Mackman, N., Moons, L., Luther, T., Gressens, P., Van Vlaenderen, I. et al. (1996) Role of tissue factor in embryonic blood vessel development. *Nature* **383**, 73–75, https://doi.org/10.1038/383073a0
243 Kishi, Y., Kondo, T., Xiao, S., Yosef, N., Gaublomme, J., Wu, C. et al. (2016) Protein C receptor (PROCR) is a negative regulator of Th17 pathogenicity. *J. Exp. Med.* **213**, 2489–2501, https://doi.org/10.1084/jem.20151118
244 Lazic, D., Sagare, A.P., Nikolakopoulou, A.M., Griffin, J.H., Vassar, R. and Zlokovic, B.V. (2019) 3K3A-activated protein C blocks amyloidogenic BACE1 pathway and improves functional outcome in mice. *J. Exp. Med.* **216**, 279–293, https://doi.org/10.1084/jem.20181035