REVIEW

New insights into pathways that determine the link between infection and thrombosis

M. Levi[1]*, T. van der Poll[1,2], M. Schultz[3,4]

[1]Department of Medicine, [2]Laboratory of Experimental Medicine and Center for Infection and Immunity Amsterdam (CINIMA), [3]Laboratory for Experimental Intensive Care and Anesthesiology, [4]Department of Intensive Care, Academic Medical Center, University of Amsterdam, the Netherlands, *corresponding author: e-mail: m.m.levi@amc.uva.nl

ABSTRACT

Severe infection is often linked to prothrombotic events. Indeed, haemostatic abnormalities are encountered in most cases of infection, ranging from an increase in sensitive markers for coagulation activation or insignificant laboratory changes to gross activation of coagulation that may result in localised thrombotic complications or disseminated intravascular coagulation. Systemic inflammation as a consequence of infection results in activation of coagulation, due to tissue factor-mediated thrombin generation, down-regulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. Pro-inflammatory cytokines, immune cells and the endothelium form the interface on which differential effects on the coagulation and fibrinolysis pathways may ensue. Conversely, activation of the coagulation system may importantly affect inflammatory responses by direct and indirect mechanisms. Apart from the general coagulation response to inflammation associated with severe infection, specific infections may cause distinct features, such as haemorrhagic fever or thrombotic microangiopathy.

KEYWORDS

Infection, inflammation, thrombosis, coagulation, endothelium, cytokines

INTRODUCTION

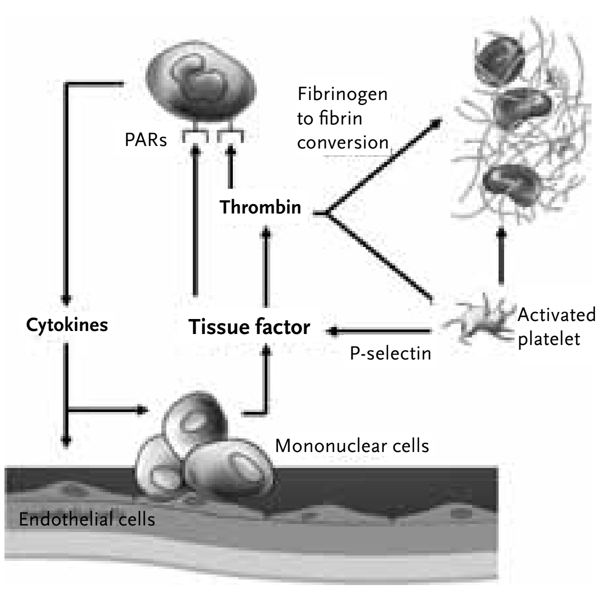
Increasing evidence points to a tight interaction between coagulation on the one hand and inflammation as a response to severe infection or chronic inflammatory states on the other hand.[1-3] In recent years the various mechanisms that play an important role in this interaction have been elucidated and this knowledge has indeed been demonstrated to be applicable for the improvement of our understanding of the pathogenesis of severe infection or chronic inflammatory states and, even more importantly, the clinical management of these patients.[4,5] In this article the mechanisms that play a role in the interaction between infection, inflammation and coagulation will be reviewed. Specific features of infectious disease-mediated effects on the coagulation system will be highlighted and the relevance for clinically relevant thrombotic manifestations is discussed.

INFECTION AND INFLAMMATION RESULT IN ACTIVATION OF COAGULATION

Acute inflammation, as a response to severe infection or trauma, results in a systemic activation of the coagulation system.[4,6] It was initially thought that this systemic activation of coagulation was a result of direct activation of the contact system of coagulation by microorganisms or endotoxin. However, in the 1990s it became apparent that cytokines played a mediatory role in the activation of coagulation and subsequent fibrin deposition and that the point of impact on the coagulation system was rather the tissue factor-factor VIIa ('extrinsic') pathway than the contact system ('intrinsic pathway').[7,8] Furthermore, the significance of impaired physiological anticoagulant pathways became increasingly clear.[9] Lastly, it was shown that impaired fibrin removal by a suppressed fibrinolytic system contributed importantly to the microvascular deposition of fibrin.

Vascular endothelial cells play a central role in all mechanisms that contribute to inflammation-induced activation of coagulation (*figure 1*). Endothelial cells respond

Figure 1. *Schematic representation of the link between infection/inflammation and coagulation*



Activated mononuclear cells and endothelial cells induce expression of tissue factor that activates platelets and the coagulation system. Activated coagulation proteases bind to protease-activated receptors (PARs), which may induce additional pro-inflammatory stimuli by releasing cytokines that target endothelial cells and mononuclear cells.

to the cytokines expressed and released by activated leucocytes but can also release cytokines themselves.[10] Furthermore, endothelial cells are able to express adhesion molecules and growth factors that may not only promote the inflammatory response further but also affect the coagulation response. However, it has recently become clear that, in addition to these mostly indirect effects of the endothelium, endothelial cells interfere directly with the initiation and regulation of fibrin formation and removal during severe infection.[11,12]

MECHANISMS OF THE INFECTION-INDUCED ACTIVATION OF COAGULATION

Inflammation-induced coagulation activation is characterised by widespread intravascular fibrin deposition, which appears to be a result of enhanced fibrin formation and impaired fibrin degradation.[1,13] Enhanced fibrin formation is caused by tissue factor-mediated thrombin generation and simultaneously occurring depression of inhibitory mechanisms, such as the protein C and S system. The impairment of endogenous thrombolysis is mainly due to high circulating levels of plasminogen activator inhibitor type 1 (PAI-1), the principal inhibitor of plasminogen activation. These derangements in coagulation and fibrinolysis are mediated by differential effects of various pro-inflammatory cytokines.[7]

Tissue factor plays a central role in the initiation of inflammation-induced coagulation.[14] Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxaemia or bacteraemia.[15,16] The vast majority of cells constitutively expressing tissue factor are found in tissues not in direct contact with blood, such as the adventitial layer of larger blood vessels. However, tissue factor comes into contact with blood when the integrity of the vessel wall is disrupted or when endothelial cells and/or circulating blood cells start expressing tissue factor. The *in vivo* expression of tissue factor seems mostly dependent on interleukin (IL)-6, as demonstrated in studies showing that inhibition of IL-6 completely abrogates tissue factor-dependent thrombin generation in experimental endotoxaemia, whereas specific inhibition of other pro-inflammatory cytokines had less or no effect.[7,17] Inflammatory cells in atherosclerotic plaques produce abundant tissue factor and upon plaque rupture there is extensive tissue factor exposure to blood.[18] In severe sepsis, mononuclear cells, stimulated by pro-inflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation.[19] Even in experimental low-dose endotoxaemia in healthy subjects, a 125-fold increase in tissue factor mRNA levels in blood monocytes can be detected.[20] A potential alternative source of tissue factor may be endothelial cells, polymorphonuclear cells, and other cell types. It is hypothesised that tissue factor from these sources is shuttled between cells through microparticles derived from activated mononuclear cells.[21] It is, however, unlikely that these cells actually synthesise tissue factor in substantial quantities.[19,22]

Upon exposure to blood, tissue factor binds to factor VIIa. The complex of tissue factor-factor VIIa catalyses the conversion of factor X to Xa, which will form the prothrombinase complex with factor Va, prothrombin (factor II) and calcium, thereby generating thrombin (factor IIa). One of the key functions of thrombin is to convert fibrinogen into fibrin. The tissue factor-factor VIIa complex can also activate factor IX, forming a tenase complex with activated factor IX and factor X, generating additional factor Xa, thereby forming an essential amplification loop. The assembly of the prothrombinase and tenase complex is markedly facilitated if a suitable phospholipid surface is available, ideally presented by activated platelets. In the setting of inflammation-induced activation of coagulation, platelets can be activated directly by endotoxin or by pro-inflammatory mediators, such as platelet activating factor. Thrombin itself is one of the strongest platelet activators *in vivo*.

Activation of platelets may also accelerate fibrin formation by another mechanism.[23] The expression of tissue factor on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin dependent reaction.[24] This effect may be the result of nuclear factor kappa B (NF-κB) activation induced by binding of activated platelets to neutrophils and mononuclear cells.[25] This cellular interaction also markedly enhances the production of IL-1b, IL-8, macrophage chemoattractant protein (MCP)-1, and tumour necrosis factor (TNF)-α.[26] The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leucocytes.

IMPAIRED REGULATORY PATHWAYS IN INFECTION AND INFLAMMATION

Procoagulant activity is regulated by three important anticoagulant pathways: antithrombin (AT), the protein C system and tissue factor pathway inhibitor (TFPI). During inflammation-induced activation of coagulation, the function of all three pathways can be impaired.[27] The serine protease inhibitor antithrombin is the main inhibitor of thrombin and factor Xa. Without heparin, AT neutralises coagulation enzymes in a slow, progressive manner.[28] Heparin induces conformational changes in AT that result in at least a 1000-fold enhancement of AT activity. Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans, such as heparan sulphates, on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes. During severe inflammatory responses, AT levels are markedly decreased owing to impaired synthesis (as a result of a negative acute phase response), degradation by elastase from activated neutrophils, and – quantitatively most importantly – consumption as a consequence of ongoing thrombin generation.[29] Pro-inflammatory cytokines can also cause reduced synthesis of glycosaminoglycans on the endothelial surface, which will also contribute to reduced AT function, since these glycosaminoglycans can act as physiological heparin-like cofactors of AT.[30] Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction.[31] There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis.[32,33] The circulating zymogen protein C is activated by the endothelial cell-bound thrombomodulin once this is activated by thrombin.[34] APC acts in concert with its co-factor protein S to proteolytically degrade the essential coagulation co-factors Va and VIIIa, and in that manner functions as an effective anticoagulant. The endothelial protein C receptor (EPCR) not only accelerates the activation of protein C several-fold, but also serves as a receptor for APC, and binding of APC to this receptor may amplify its anticoagulant and anti-inflammatory effects.[35] A recent study has demonstrated that exposure of cultured endothelial cells to APC results in the release of microparticles that contain EPCR.[36] but the relevance of that observation for coagulation or inflammation is not yet clear. In patients with severe inflammation, the protein C system is malfunctioning at virtually all levels. First, plasma levels of the zymogen protein C are low or very low, due to impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase.[37-39] Furthermore, a significant down-regulation of thrombomodulin, caused by pro-inflammatory cytokines such as TNF-α and IL-1, has been demonstrated, resulting in diminished protein C activation.[40,41] Low levels of free protein S may further compromise an adequate function of the protein C system. In plasma, 60% of the co-factor protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP as a consequence of the acute phase reaction in inflammatory diseases may result in a relative protein S deficiency, which further contributes to a procoagulant state during sepsis. Although it has been shown that the β-chain of C4bBP (which mainly governs the binding to protein S) is largely unaffected during the acute phase response,[42] support for this hypothesis comes from studies showing that the infusion of C4bBP in combination with a sublethal dose of *Escherichia coli* (*E. coli*) into baboons resulted in a lethal response with severe organ damage due to disseminated intravascular coagulation (DIC).[43] Finally, but importantly, in sepsis the EPCR has shown to be down-regulated, which may further negatively affect the function of the protein C system. Apart from these effects, sepsis may cause a resistance toward APC by other mechanisms, which are partly dependent on a sharp increase in factor VIII levels (released from endothelial cells), but partly occur by yet unidentified mechanisms.[45] In experimental models of severe infection fibrinolysis is activated, demonstrated by an initial activation of plasminogen activation, followed by a marked impairment caused by the release in blood of PAI-1.[16,46,47] The latter inhibitor strongly inhibits fibrinolysis causing a net procoagulant situation. The molecular basis is cytokine-mediated activation of vascular endothelial cells; TNFα and IL-1 decreased free tissue plasminogen activator (tPA) and increased PAI-1 production, TNFa increased total urokinase type plasminogen activator (uPA) production in endothelial cells.[48,49] Endotoxin and TNFα stimulated PAI-1 production in liver, kidney, lung and adrenals of mice. The net procoagulant state is illustrated by a late rise in fibrin breakdown fragments after *E. coli* challenge of baboons. Experimental data also indicate that the fibrinolytic mechanism is active in clearing fibrin from organs and circulation. Endotoxin-induced fibrin formation in kidneys and adrenals was most dependent on a decrease in uPA.[50] PAI-1 knockout mice challenged with endotoxin did not develop thrombi in the kidney in contrast to wildtype animals.[49] Endotoxin administration to mice with a functionally inactive thrombomodulin gene (TMProArg mutation) and defective protein C activator cofactor function caused fibrin plugs in the pulmonary circulation, while wildtype animals did not develop macroscopic fibrin.[51] This phenomenon proved to be temporary, with detectable thrombi at four hours after endotoxin, and disappearance of clots at 24 hours in animals sacrificed at that time point. These experiments demonstrate that fibrinolytic action is required to reduce the extent of intravascular fibrin formation.

Fibrinolytic activity is markedly regulated by PAI-1, the principal inhibitor of this system. Recent studies have shown that a functional mutation in the PAI-1 gene, the 4G/5G polymorphism, not only influenced the plasma levels of PAI-1, but was also linked to clinical outcome of meningococcal septicaemia. Patients with the 4G/4G genotype had significantly higher PAI-1 concentrations in plasma and an increased risk of death.[52] Further investigations demonstrated that the PAI-1 polymorphism did not influence the risk of contracting meningitis as such, but probably increased the likelihood of developing septic shock from meningococcal infection.[53] These studies are the first evidence that genetically determined differences in the level of fibrinolysis influences the risk of developing complications of a Gram-negative infection. In other clinical studies in cohorts of patients with DIC, high plasma levels of PAI-1 were one of the best predictors of mortality.[54,55] These data suggest that activation of coagulation contributes to mortality in this situation, but as indicated earlier, the fact that PAI-1 is an acute phase protein, a higher plasma concentration may also be a marker of disease rather than a causal factor. Interestingly, platelet α-granules contain large quantities of PAI-1 and release PAI-1 upon their activation. Since platelets become activated in case of severe inflammation and infection, this may further increase the levels of PAI-1 and contribute to the fibrinolytic shut-off.

INFECTION-INDUCED THROMBOSIS AND VASCULAR COMPLICATIONS

Apart from the generalised response upon systemic inflammation as discussed above, specific infections may result in thrombohaemorrhagic syndromes, haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) or vasculitis.[56,57] Symptoms and signs may be dominated by bleeding, thrombosis, or both.[1,58,59] Clinically overt infection-induced activation of coagulation may occur in 30 to 50% of patients with Gram-negative sepsis.[60] Contrary to widely held belief, this may appear as common in patients with Gram-positive sepsis as in those with Gram-negative sepsis.[60,61] Activation of the coagulation system has also been documented for non-bacterial pathogens, i.e. viruses,[62,63] protozoa (malaria),[64,65] fungi[66] and spirochetes.[67]

Viral and bacterial infections may result in an enhanced risk for local thromboembolic disease, i.e. deep venous thrombosis or pulmonary embolism. In a thromboembolic prevention study of low-dose subcutaneous standard heparin for hospitalised patients with infectious diseases, morbidity due to thromboembolic disease was significantly reduced in the heparin group compared with the group receiving no prophylaxis. There was, however, no beneficial effect of prophylaxis on mortality due to thromboembolic complications.[68] In chronic viral diseases, such as cytomegalovirus (CMV) or human immuno-deficiency virus (HIV) infection, the risk of thromboembolic complications is relatively low.[69-71] Common infections, such as influenza and other forms of upper respiratory tract infections, have been shown to not only increase systemic levels of haemostatic proteins, but also to affect the incidence of pulmonary embolism, albeit to a modest extent.[72,73] Also, these conditions seem to predispose for the occurrence of ischaemic stroke.[74] A recent paper points to the fact that the enhanced thrombotic risk may be related to inflammation, either occurring on itself (e.g. as a consequence of an autoimmune disorder) or related to infection.[75]

Viral haemorrhagic fever is complicated by DIC in the most severe cases.[76-78] DIC is not frequently encountered in other viral infections but has been reported in cases of infection with rotavirus,[79,80] varicella, rubella, rubeola and influenza.[81-84] TTP and HUS, triggered by a viral or bacterial infection,[56,85] frequently lead to bleeding symptoms, but also platelet and fibrin thrombi may be generated in various organs, leading to prominent symptoms with organ dysfunction. In specific infections, such as viral haemorrhagic fever, bleeding complications are prominent.[76,77] In other viral and bacterial infections associated with TTP or HUS, bleeding is also often the prominent and presenting symptom.[55] Bacterial and viral infections may result in a vasculitis-like syndrome with either bleeding manifestations or ischaemic injury.[86-88] Vasculitis is a well-documented phenomenon in CMV infection,[89,90] occurring predominantly in the vasculature of the gastrointestinal tract where it causes colitis,[91,92] the central nervous system where it causes cerebral infarction,[93,94] and the skin where it results in petechiae, purpura papules, localised ulcers or a diffuse maculopapular eruption.[95] HIV infection may be accompanied by vasculitis syndromes, e.g. polyarteriitis nodosa, Henoch-Schönlein purpura and leucocytoclastic vasculitis.[96-98] Hepatitis B and C infection may cause polyarteritis-like vasculitis.[99,100] Parvovirus B19 has been suggested to be associated with vasculitis-like syndromes including Kawasaki disease, polyarteritis nodosa and Wegener's granulomatosis.[101-103]

THERAPEUTIC IMPLICATIONS

Anticoagulant therapy in patients with severe infection remains controversial. Experimental studies have shown that heparin can at least partly inhibit the activation of coagulation in severe sepsis and other infections. However, a beneficial effect of heparin on clinically important outcome events in patients with DIC has not been demonstrated in controlled clinical trials. Also, the safety of heparin treatment is debatable in patients with haemorrhagic complications of infection, such as in some viral diseases or in DIC, who are prone to bleeding.[104] A large trial in patients with severe sepsis showed a slight but non-significant benefit of low-dose heparin on 28-day mortality in patients with severe sepsis and no major safety concerns.[105] There is general consensus that administration of heparin is beneficial in some categories of infection-related procoagulant states. Heparin is obviously indicated for treating thromboembolic complications in large vessels in patients with inflammation and infection. Heparin administration may be helpful in patients with acute DIC when intensive blood component replacement fails to improve excessive bleeding or when thrombosis threatens to cause irreversible tissue injury (e.g., acute cortical necrosis of the kidney or digital gangrene).

Theoretically, the most logical anticoagulant agent to use in the setting of hypercoagulability in the setting of infection or inflammation is directed against tissue factor activity. Potential agents include recombinant TFPI, inactivated factor VIIa, and recombinant nematode anticoagulant protein c2 (NAPc2), a potent and specific inhibitor of the ternary complex of TF/factor VIIa and factor Xa. Phase II trials of recombinant TFPI in patients with sepsis showed promising results but phase III trials in patients with severe sepsis or severe pneumonia and organ failure did not show an overall survival benefit in patients who were treated with TFPI.[106] Recombinant human soluble thrombomodulin binds to thrombin to form a complex that inactivates thrombin's coagulant activity and activates protein C and, thus, is a potential drug for the treatment of patients with DIC. In a phase III randomised double-blind clinical trial in patients with DIC, administration of the soluble thrombomodulin had a significantly better effect on bleeding manifestations and coagulation parameters than heparin. Currently ongoing trials with soluble thrombomodulin focus on DIC, organ failure and mortality rate.

CONCLUSION

There is a tight link between infection and inflammation on the one hand and activation of coagulation and venous and arterial thrombosis on the other hand. Pro-inflammatory cytokines are crucial in mediating these effects. The interaction between inflammation and coagulation involves significant cross-talk between the systems and seems to occur at the interface formed by endothelial cells. Several mechanisms contribute to an enhanced risk of both venous thromboembolism and accelerated atherosclerosis in patients with infections and (chronic) inflammation. Although it is likely that anticoagulant treatment is important to prevent infection- and inflammation-associated thrombotic complications, clinical evidence of efficacy and safety of this approach is still limited.

REFERENCES

1 Levi M, ten Cate H. Disseminated intravascular coagulation. N Engl J Med. 1999;341:586-92.

2 Wheeler AP, Bernard GR. Treating patients with severe sepsis. N Engl J Med. 1999;340:207-14.

3 Levi M, Keller TT, van Gorp E, ten Cate H. Infection and inflammation and the coagulation system. Cardiovasc Res. 2003;60:26-39.

4 Levi M, Schultsz M, van der Poll T. Infection and inflammation as a risk factor for thrombosis. Sem Thromb Hemostas. 2012; in press.

5 Levi M, van der Poll T. Inflammation and coagulation. Crit Care Med. 2010;38:S26-S34.

6 Esmon CT, Fukudome K, Mather T, et al. Inflammation, sepsis, and coagulation. Haematologica. 1999;84:254-9.

7 Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest. 1997;27:3-9.

8 Osterud B, Bjorklid E. The tissue factor pathway in disseminated intravascular coagulation. Semin Thromb Hemost. 2001;27:605-17.

9 Taylor FB. Response of anticoagulant pathways in disseminated intravascular coagulation. Semin Thromb Hemost. 2001;27:619631.

10 ten Cate JW, van der Poll T, Levi M, et al. Cytokines: triggers of clinical thrombotic disease. Thromb Haemost. 1997;78:415-9.

11 Aird WC. Vascular bed-specific hemostasis: role of endothelium in sepsis pathogenesis. Crit Care Med. 2001;29:S28-S34.

12 Levi M, ten Cate H, van der Poll T. Endothelium: interface between coagulation and inflammation. Crit Care Med. 2002;30:S220-S224.

13 Vallet B. Microthrombosis in sepsis. Minerva Anestesiol. 2001;67:298-301.

14 Levi M, van der Poll T, ten Cate H. Tissue factor in infection and severe inflammation. Semin Thromb Hemost. 2006;32:33-9.

15 Taylor FBJ, Chang A, Ruf W, et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock. 1991;33:127-34.

16 Levi M, ten Cate H, Bauer KA, et al. Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. J Clin Invest. 1994;93:114-20.

17 van der Poll T, Levi M, Hack CE, et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J Exp Med. 1994;179:1253-9.

18 Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med. 2002;8:1257-62.

19 Osterud B, Rao LV, Olsen JO. Induction of tissue factor expression in whole blood – lack of evidence for the presence of tissue factor expression on granulocytes. Thromb Haemost. 2000;83:861-7.

20 Franco RF, de Jonge E, Dekkers PE, et al. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. Blood. 2000;96:554-9.

21 Rauch U, Bonderman D, Bohrmann B, et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. Blood. 2000;96:170-5.

22 Osterud B, Bjorklid E. Sources of tissue factor. Semin Thromb Hemost. 2006;32:11-23.

23 Lowenberg EC, Meijers JC, Levi M. Platelet-vessel wall interaction in health and disease. Neth J Med. 2010;68:242-51.

24 Osterud B. Tissue factor expression by monocytes: regulation and pathophysiological roles. Blood Coagul Fibrinolysis. 1998;9 Suppl 1:S9-14.

25 Furie B, Furie BC. Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. Trends Mol Med. 2004;10:171-8.

26 Neumann FJ, Marx N, Gawaz M, et al. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation. 1997;95:2387-94.

27 Levi M, van der Poll T. The role of natural anticoagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients. Semin Thromb Hemost. 2008;34:459-68.

28 Levi M. Antithrombin in sepsis revisited. Crit Care. 2005;9:624-5.

29 Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation. 2004;109:2698-704.

30 Kobayashi M, Shimada K, Ozawa T. Human recombinant interleukin-1 beta- and tumor necrosis factor alpha-mediated suppression of heparin-like compounds on cultured porcine aortic endothelial cells. J Cell Physiol. 1990;144:383-90.

31 Levi M, van der Poll T. Recombinant human activated protein C: current insights into its mechanism of action. Crit Care. 2007;11 Suppl 5:S3:S3.

32 Esmon CT. Role of coagulation inhibitors in inflammation. Thromb Haemost. 2001;86:51-6.

33 Levi M, de Jonge E, van der Poll T. Rationale for restoration of physiological anticoagulant pathways in patients with sepsis and disseminated intravascular coagulation. Crit Care Med. 2001;29:S90-S94.

34 Esmon CT. The regulation of natural anticoagulant pathways. Science. 1987;235:1348-52.

35 Esmon CT. The endothelial cell protein C receptor [Review.] Thromb Haemost. 2000;83:639-43.

36 Perez-Casal M, Downey C, Fukudome K, Marx G, Toh CH. Activated protein C induces the release of microparticle-associated endothelial protein C receptor. Blood. 2005;105:1515-22.

37 Mesters RM, Helterbrand J, Utterback BG, et al. Prognostic value of protein C concentrations in neutropenic patients at high risk of severe septic complications. Crit Care Med. 2000;28:2209-16.

38 Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol. 1992;262:C445-C452.

39 Eckle I, Seitz R, Egbring R, Kolb G, Havemann K. Protein C degradation in vitro by neutrophil elastase. Biol Chem Hoppe Seyler. 1991;372:1007-13.

40 Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med. 1986;163:740-5.

41 Faust SN, Levin M, Harrison OB, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. N Engl J Med. 2001;345:408-16.

42 Garcia de Frutos P, Alim RI, Hardig Y, et al. Differential regulation of alpha and beta chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. Blood. 1994;84:815-22.

43 Taylor FBJ, Dahlback B, Chang AC, et al. Role of free protein S and C4b binding protein in regulating the coagulant response to Escherichia coli. Blood. 1995;86:2642-52.

44 Taylor FBJ, Stearns-Kurosawa DJ, Kurosawa S, et al. The endothelial cell protein C receptor aids in host defense against Escherichia coli sepsis. Blood. 2000;95:1680-6.

45 De Pont AC, Bakhtiari K, Hutten BA, et al. Endotoxaemia induces resistance to activated protein C in healthy humans. Br J Haematol. 2006;134:213-9.

46 Biemond BJ, Levi M, ten Cate H, et al. Plasminogen activator and plasminogen activator inhibitor I release during experimental endotoxaemia in chimpanzees: effect of interventions in the cytokine and coagulation cascades. Clin Sci (Colch). 1995;88:587-94.

47 Biemond BJ, Friederich PW, Koschinsky ML, et al. Apolipoprotein(a) attenuates endogenous fibrinolysis in the rabbit jugular vein thrombosis model in vivo. Circulation. 1997;96:1612-5.

48 Schleef RR, Bevilacqua MP, Sawdey M, et al. Cytokine activation of vascular endothelium. Effects on tissue-type plasminogen activator and type 1 plasminogen activator inhibitor. J Biol Chem. 1988;263:5797-803.

49 Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. J Clin Invest. 1991;88:1346-53.

50 Yamamoto K, Loskutoff DJ. Fibrin deposition in tissues from endotoxin-treated mice correlates with decreases in the expression of urokinase-type but not tissue-type plasminogen activator. J Clin Invest. 1996;97:2440-51.

51 ten Cate H. Pathophysiology of disseminated intravascular coagulation in sepsis. Crit Care Med. 2000;S9-S11.

52 Hermans PW, Hibberd ML, Booy R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. Lancet. 1999;354:556-60.

53 Westendorp RG, Hottenga JJ, Slagboom PE. Variation in plasminogen-activator-inhibitor-1 gene and risk of meningococcal septic shock. Lancet. 1999;354:561-3.

54 Brandtzaeg P, Joo GB, Brusletto B, Kierulf P. Plasminogen activator inhibitor 1 and 2, alpha-2-antiplasmin, plasminogen, and endotoxin levels in systemic meningococcal disease. Thromb Res. 1990;57:271-8.

55 Mesters RM, Florke N, Ostermann H, Kienast J. Increase of plasminogen activator inhibitor levels predicts outcome of leukocytopenic patients with sepsis. Thromb Haemost. 1996;75:902-7.

56 Zimmerhackl LB, Rosales A, Hofer J, et al. Enterohemorrhagic Escherichia coli O26:H11-Associated Hemolytic Uremic Syndrome: Bacteriology and Clinical Presentation. Semin Thromb Hemost. 2010;36:586-93.

57 Karpman D, Sartz L, Johnson S. Pathophysiology of typical hemolytic uremic syndrome. Semin Thromb Hemost. 2010;36:575-85.

58 van Gorp E, Suharti C, ten Cate H, et al. Review: infectious diseases and coagulation disorders. J Infect Dis. 1999;180:176-86.

59 Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature. 2000;406:782-87.

60 Anas AA, Wiersinga WJ, de Vos AF, van der Poll T. Recent insights into the pathogenesis of bacterial sepsis. Neth J Med. 2010;68:147-52.

61 Vos FJ, Bleeker-Rovers CP, Sturm PD, et al. Endocarditis: effects of routine echocardiography during Gram-positive bacteraemia. Neth J Med. 2011;69:335-40.

62 Bhamarapravati N. Hemostatic defects in dengue hemorrhagic fever. Rev Infect Dis. 1989;11 Suppl 4:S826-S829.

63 Heller MV, Marta RF, Sturk A, et al. Early markers of blood coagulation and fibrinolysis activation in Argentine hemorrhagic fever. Thromb Haemost. 1995;73:368-73.

64 Mohanty D, Ghosh K, Nandwani SK, et al. Fibrinolysis, inhibitors of blood coagulation, and monocyte derived coagulant activity in acute malaria. Am J Hematol. 1997;54:23-9.

65 Chuttani K, Tischler MD, Pandian NG, et al. Diagnosis of cardiac tamponade after cardiac surgery: relative value of clinical, echocardiographic, and hemodynamic signs. Am Heart J. 1994;127:913-8.

66 Fera G, Semeraro N, De Mitrio V, Schiraldi O. Disseminated intravascular coagulation associated with disseminated cryptococcosis in a patient with acquired immunodeficiency syndrome. Infection. 1993;21:171-3.

67 Schroder S, Spyridopoulos I, Konig J, et al. Thrombotic thrombocytopenic purpura (TTP) associated with a Borrelia burgdorferi infection. Am J Hematol. 1995;50:72-3.

68 Gardlund B. Randomised, controlled trial of low-dose heparin for prevention of fatal pulmonary embolism in patients with infectious diseases. The Heparin Prophylaxis Study Group.[see comments] Lancet. 1996;347:1357-61.

69 Laing RB, Brettle RP, Leen CL. Venous thrombosis in HIV infection. [editorial] Int J STD AIDS. 1996;7:82-5.

70 Jenkins RE, Peters BS, Pinching AJ. Thromboembolic disease in AIDS is associated with cytomegalovirus disease.[letter] AIDS. 1991;5:1540-2.

71 Drancourt M, George F, Brouqui P, Sampol J, Raoult D. Diagnosis of Mediterranean spotted fever by indirect immunofluorescence of Rickettsia conorii in circulating endothelial cells isolated with monoclonal antibody-coated immunomagnetic beads. J Infect Dis. 1992;166:660-3.

72 Keller TT, van Wissen M, Mairuhu AT, van Doornum GJ, Brandjes DP. Acute respiratory tract infections in elderly patients increase systemic levels of hemostatic proteins. J Thromb Haemost. 2007;5:1567-9.

73 van Wissen M, Keller TT, Ronkes B, et al. Influenza infection and risk of acute pulmonary embolism. Thromb J. 2007;5:16.

74 Grau AJ, Urbanek C, Palm F. Common infections and the risk of stroke. Nat Rev Neurol. 2010;6:681-94.

75 Zoller B, Li X, Sundquist J, Sundquist K. Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. Lancet. 2011.

76 Hayes EB, Gubler DJ. Dengue and dengue hemorrhagic fever.[Review] Pediatr Infect Dis J. 1992;11:311-7.

77 Sumarmo, Wulur H, Jahja E et al. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. Bull World Health Organ. 1983;61:693-701.

78 Kuberski T, Rosen L, Reed D, Mataika J. Clinical and laboratory observations on patients with primary and secondary dengue type 1 infections with hemorrhagic manifestations in Fiji. Am J Trop Med Hyg. 1977;26:775-83.

79 Limbos MA, Lieberman JM. Disseminated intravascular coagulation associated with rotavirus gastroenteritis: report of two cases.[see comments] Clin Infect Dis. 1996;22:834-36.

80 Anderson DR, Harrison L, Hirsh J. Evaluation of a portable prothrombin time monitor for home use by patients who require long-term oral anticoagulant therapy. Arch Intern Med. 1993;153:1441-7.

81 McKay DG, Margaretten W. Disseminated intravascular coagulation in virus diseases. Arch Intern Med. 1967;120:129-52.

82 Linder M, Muller-Berghaus G, Lasch HG, Gagel C. Virus infection and blood coagulation. Thromb Diath Haemorrh. 1970;23:1-11.

83 Anderson DR, Schwartz J, Hunter NJ, et al. Varicella hepatitis: a fatal case in a previously healthy, immunocompetent adult. Report of a case, autopsy, and review of the literature. Arch Intern Med. 1994;154:2101-6.

84 Clemens R, Pramoolsinsap C, Lorenz R, et al. Activation of the coagulation cascade in severe falciparum malaria through the intrinsic pathway. Br J Haematol. 1994;87:100-5.

85 Badesha PS, Saklayen MG. Hemolytic uremic syndrome as a presenting form of HIV infection. Nephron. 1996;72:472-5.

86 Ackerman AB, Chongchitnant N, Sanchez J, Guo Y. Inflammatory diseases; Histologic diagnosis of inflammatory skin diseases. Williams and Wilkins. 1997;170-86.

87 Lie JT. Vasculitis associated with infectious agents. Curr Opin Rheumatol. 1996;8:26-9.

88 Guillevin L, Lhote F, Gherardi R. The spectrum and treatment of virus-associated vasculitides. Curr Opin Rheumatol. 1997;9:31-6.

89 Golden MP, Hammer SM, Wanke CA, Albrecht MA. Cytomegalovirus vasculitis. Case reports and review of the literature. Medicine. 1994;73:246-55.

90 Ho DD, Rota TR, Andrews CA, Hirsch MS. Replication of human cytomegalovirus in endothelial cells. J Infect Dis. 1984;150:956-7.

91 Goodman MD, Porter DD. Cytomegalovirus vasculitis with fatal colonic hemorrhage. Arch Pathol Lab Med. 1973;96:281-4.

92 Foucar E, Mukai K, Foucar K, et al. Colon ulceration in lethal cytomegalovirus infection. Am J Clin Pathol. 1981;76:788-801.

93 Booss J, Dann PR, Winkler SR, et al. Mechanisms of injury to the central nervous system following experimental cytomegalovirus infection. Am J Otolaryngol. 1990;11:313-7.

94 Koeppen AH, Lansing LS, Peng SK, Smith RS. Central nervous system vasculitis in cytomegalovirus infection. J Neurol Sci. 1981;51:395-410.

95 Lin CS, Penha PD, Krishnan MN, Zak FG. Cytomegalic inclusion disease of the skin. Arch Dermatol. 1981;117:282-4.

96 Libman BS, Quismorio FPJ, Stimmler MM. Polyarteritis nodosa-like vasculitis in human immunodeficiency virus infection. J Rheumatol. 1995;22:351-5.

97 Calabrese LH. Vasculitis and infection with the human immunodeficiency virus. Rheum Dis Clin North Am. 1991;17:131-47.

98 Gherardi R, Belec L, Mhiri C, et al. The spectrum of vasculitis in human immunodeficiency virus-infected patients. A clinicopathologic evaluation. Arthritis Rheum. 1993;36:1164-74.

99 Sergent JS, Lockshin MD, Christian CL, et al. Vasculitis with hepatitis B antigenemia: long-term observation in nine patients. Medicine. 1976;55:1-18.

100 Carson CW, Conn DL, Czaja AJ, et al. Frequency and significance of antibodies to hepatitis C virus in polyarteritis nodosa. J Rheumatol. 1993;20:304-9.

101 Leruez-Ville M, Lauge A, Morinet F, et al. Polyarteritis nodosa and parvovirus B19. Lancet. 1994;344:263-4.

102 Nikkari S, Mertsola J, Korvenranta H, et al. Wegener's granulomatosis and parvovirus B19 infection. Arthritis Rheum. 1994;37:1707-8.

103 Yoto Y, Kudoh T, Haseyama K, et al. Human parvovirus B19 infection in Kawasaki disease.[letter; comment] Lancet. 1994;344:58-9.

104 Levi MM, Eerenberg E, Lowenberg E, Kamphuisen PW. Bleeding in patients using new anticoagulants or antiplatelet agents: risk factors and management. Neth J Med. 2010;68:68-76.

105 Levi M, Levy M, Williams MD, et al. Prophylactic heparin in patients with severe sepsis treated with drotrecogin alfa (activated). Am J Respir Crit Care Med. 2007;176:483-90.

106 Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. JAMA. 2003;290:238-47.