Review

Infections and endothelial cells

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Abstract

Systemic infection by various pathogens interacts with the endothelium and may result in altered coagulation, vasculitis and atherosclerosis. Endothelium plays a role in the initiation and regulation of both coagulation and fibrinolysis. Exposure of endothelial cells may lead to rapid activation of coagulation via tissue factor (TF) expression and the loss of anticoagulant properties by impairment of antithrombin III, TF pathway inhibitor (TFPI) and the protein C system. Endothelial-derived plasminogen activator inhibitor (PAI) is essential for the regulation of fibrinolysis and impaired endothelial function leads to imbalance in fibrinolysis, resulting in a procoagulant state. The interaction between inflammation and coagulation, soluble adhesion molecules and circulation endothelial cells is important in the pathogenesis of an unbalanced haemostatic system. Rather than being a unidirectional relationship, the interaction between inflammation and coagulation appears to be significant. In the crosstalk, the endothelium is playing a pivotal role.

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1. Introduction

The vascular endothelium exerts a number of important functions in order to maintain adequate blood supply to vital organs. These functions include prevention of coagulation, regulation of vascular tonus, orchestration of the migration of blood cells by the expression of adhesion molecules, regulation of vasopermeability and production of chemotactant compounds. Injury or activation of endothelial cells in response to inflammation could grossly alter these functions. During severe inflammation leading to shock, the role of endothelial cells in the process of increased vascular leakage and thrombopathy seems to be crucial [1]. The endothelial cell surface changes from an anti- into a prothrombotic surface and the thromboresistance of the cardiovascular system is further decreased by the impairment of endothelium-dependent relaxation [2]. Endothelial cells are able to express a wide variety of cytokines and factors that alter the haemostatic balance in a prothrombotic state. In addition to the indirect effects of adhesion molecules, growth factor and cytokines, endothelial cells interfere directly with the initiation, regulation and modulation of coagulation (Fig. 1).

Tissue factor (TF), as principal initiator of this inflammation-induced thrombin generation [3,4] is expressed by endothelial cells [5], which in addition to the decreased production of antithrombin and protein C, leads to a prothrombotic state [6]. Furthermore, during sepsis the activated coagulation system and formation of fibrin is not counteracted by fibrinolysis, because of an impaired endo-

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Fig. 1. Endothelial cells (EC) and infection. Under normal conditions endothelial cells contain anticoagulant agents, such as thrombomodulin, heparan sulphate (HP) and plasminogen activator. Stimulation by pathogens and/or cytokines activates the endothelial cells. Tissue factor (TF) and von Willebrand factor (vWF) are expressed. Subsequently coagulation factors are activated and fibrin is formed. Furthermore normal antithrombotic properties are lost: antithrombin (AT), activated protein C (APC) and fibrinolysis. Activation of endothelial cells results in a procoagulant state.

Thus, an activated or defective endothelium influences all three major pathways of the coagulation system: tissue factor mediated thrombin generation, dysfunction of anticoagulant pathways and blocked fibrinolysis. In this review we will discuss current insights into the major mechanisms involving the endothelium for the initiation and regulation of the coagulation system in acute and chronic infectious diseases.

2. Clinical aspects

2.1. Acute infections and altered coagulation system

During acute infections the endothelium may become activated by pathogens or indirectly via inflammatory mediators. Depending on the pathogen, there are several mechanisms by which the endothelium is challenged [1]. Direct activation by bacteria, viruses and other pathogens or indirect activation of the endothelium may result in altered coagulation and fibrinolytic systems [8]. In sepsis with gram negative bacteria, activation by endotoxins is characteristic. Invasion of endothelial cells by a number of viruses is common, for example (para-)influenza, adenoviruses, herpes simplex virus (HSV), polio virus, echovirus, measles virus, mumps virus, cytomegalovirus (CMV), human T-cell leukaemia virus type-1 (HTLV-1) and human immunodeficiency virus (HIV) [8,9]. Infection of endothelial cells has been demonstrated for haemorrhagic fevers (HF) caused by Dengue, Marburg, Ebola, Hantaan and Lassa HF as well [8]. HIV infection alters the coagulation system by impairment of heparin co-factor II [10]. Not endothelium-related procoagulant actions of HIV are the prothrombinase complex assembly on the viral surface [11] and a reduced protein S system [12]. Leptospirosis, especially Weil’s syndrome, may be presented with haemoptysis, epistaxis, intestinal bleeding, adrenal bleeding, haematuria, and even subarachnoid haemorrhage [13]. The pathogenesis may be either primary activation of coagulation or diffuse vasculitis, resulting in bleeding or ischaemia of the vascularized tissue. The enhanced coagulability of CMV may be mediated by the prothrombinase complex assembly on the CMV surface [14], but this action is not endothelial dependent. This observation suggests that the CMV surface contains the necessary procoagulant phospholipids for assembly of the coagulation enzyme complex leading to thrombin generation.

Infectious agents have been linked with coronary artery disease and myocardial infarction. Different authors have suggested a link between Coxsackie B virus and coronary
heart disease [15,16]. In acute coronary syndromes the levels of circulation inflammatory markers, such as CRP, serum amyloid A and interleukin (IL)-6, are elevated [17–19]. A recent investigation of a large number of randomly selected patients demonstrated an increase in enterovirus-specific antibodies at the time of diagnosis of myocardial infarction, thus suggesting that this virus may trigger acute coronary syndromes [20].

2.1.1. Disseminated intravascular coagulation

Endothelial injury by endotoxines [21] is considered to be the primary event of disseminated intravascular coagulation (DIC), an acquired disorder in which the coagulation system is activated. Furthermore, the release of endotoxines activates the extrinsic route of the coagulation system [22]. Several clinical conditions, i.e. bacterial and severe viral infections leading to DIC, may alter the coagulation system by activation of TF-mediated pathways [23,24]. The infection results in activation of endothelium and platelets and the conversion of fibrinogen to fibrin. Consumption of platelets, coagulation factors and activation of the fibrinolytic system are hallmarks of the clinical syndrome. Patients with DIC may present with manifest thrombo-embolic disease or the clinically less apparent microvascular thrombosis. Bleeding, thrombosis, or both may dominate symptoms and signs.

2.1.2. Vasculitis

Vasculitis, which may be triggered by infection, is characterized by local or more generalized vascular changes, resulting from infarction secondary to occlusion by thrombi of the lumen of small blood vessels in the upper part of the dermis. Vasculitis has been documented in association with bacterial and viral infections [25]. Coronary arteritis was shown during experimental infections with Coxsackie B4 virus in mice [26]. Different viruses have been associated with graft arteriopathy [27]. Recent research has demonstrated a link between viral infections, particularly adenovirus, and coronary vasculopathy leading to graft loss after heart transplantation [28]. HIV infection is linked with polyarteritis nodosa, Henoch–Schonlein purpura and leukocytoclastic vasculitis [29]. Polyarteritis nodosa may occur also in hepatitis B and C [30,31] and CMV infections [32]. Vasculitis-like syndromes, including Kawasaki disease, polyarteritis nodosa, and Wegener’s granulomatosis has been associated with Parvovirus B19 [33,34]. None of these syndromes, DIC or vasculitis, are specific for a certain pathogen, and their occurrence depends on factors such as virulence of the pathogen, the patient’s prior condition, source and size of the inoculums, and availability of antimicrobial treatment.

2.2. Atherosclerosis

The clinical consequences of chronic infections for the development of thrombotic complications, atherosclerosis and cardiovascular complications are highly significant. Recent research has focused on unravelling the mechanisms of chronic infection leading to atherosclerosis. Pathogens, including viruses such as H. simplex (HSV), hepatitis A (HAV) and cytomegalovirus (CMV) and bacteria such as C. pneumoniae and H. pylori and peridontitis [35] can be linked with vascular pathology. The process of the influx of blood leukocytes and plaque formation has been described for several pathogens, i.e. C. pneumoniae and H. pylori [36,37]. In chronic infections activation of the endothelium may result in long-term complications such as atherosclerosis and subsequent cardiovascular disease [38]. Serologically, several infectious agents have been associated with cardiovascular disease, including bacteria such as C. pneumoniae and H. pylori and viruses such as CMV, Coxsackie B virus, enteroviruses, HSV and Epstein–Barr virus. Furthermore, there is some evidence that the presence of antibodies directed against C. pneumoniae and CMV are associated with an increased risk of future cardiovascular events [39]. Increased titres of antibodies against C. pneumoniae have been used as a predictor of further adverse events in patients who have had a myocardial infarction [40]. In fact, a cumulative ‘pathogen burden’ seems to lead to atherogenesis and cardiovascular disease. Nevertheless epidemiological studies on the relationship between chronic infections and atherosclerosis have yielded mixed results and the pathogenesis has not yet been clarified.

3. (Patho)physiology of endothelial activation during inflammation

Endothelial cells have a primary function in combating blood clotting and thrombosis (Table 1). In pathological conditions prothrombotic properties are of major importance. During inflammation, endothelial cells are involved in all coagulation mechanisms leading to a procoagulant state: TF mediated initiation of the coagulation cascade, disturbed regulation mechanisms and impaired fibrinolysis.

3.1. Tissue factor pathway

TF expression on the surface of activated endothelial cells, monocytes and macrophages can be induced in vitro by a number of proinflammatory mediators and several other compounds, including cytokines, C-reactive protein and advanced glycosilated endproducts [5]. Some evidence is emerging that endothelial cells also play an important role in the generation of TF during severe infection. The main route for activation of the coagulation cascade during sepsis is the TF-factor VIIa pathway [41] (Fig. 1). In primates, antibodies directed against TF or factor VIIa or treatment with TF pathway inhibitor (TFPI) prevent activation of the common pathway [7]. Factor VIIa, in a complex with TF catalyzes the conversion of factors IX and X [42].
Table 1

<table>
<thead>
<tr>
<th>Action</th>
<th>Anticoagulant properties</th>
<th>Fibrinolysis</th>
<th>Antiplatelet aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin binding to heparan sulphate</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Synthesis and release of protein S</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thrombomodulin expression</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor (TFPI)</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tissue-type plasminogen activator (tPA)</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>Urokinase plasminogen activator (uPA)</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>Induced synthesis of plasminogen activator inhibitor (PAI-1)</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>Prostacyclin synthesis</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Synthesis of nitric oxide (NO)</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Surface heparan sulphate</td>
<td>Yes</td>
<td>–</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Factors IXa and Xa enhance the activation of factor X and prothrombin, respectively. Although contact activation does not seem to play an essential role in activation of the coagulation cascade, blocking of the contact activation system by the administration of monoclonal antibodies against factor XIIa could prevent lethal hypotension [43]. This effect is most probably generated by kinins [43] and the nitric oxide mediated vasodilatation [44]. Moreover, the contact system seems to play an independent role in the activation of the fibrinolytic system [45].

3.2. Regulatory properties of the endothelium

Under normal conditions endothelial cells exhibit major antithrombotic properties. Several mechanisms are responsible for this homeostasis (Fig. 1). Antiplatelet function seems to be intrinsic to the plasma membrane of endothelium. When platelets are activated, aggregation to intact endothelium is inhibited by prostacyclin and nitric oxide. Their synthesis in endothelial cells is stimulated by ADP, thrombin, cytokines and other factors produced during coagulation [46]. Thrombin generation is limited by antithrombin, the proteins C system and TF pathway inhibitor (TFPI). The fibrinolytic system is another key element in the regulation of fibrin deposition. Fibrinolysis is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) after their synthesis and release from the endothelial cell system. These activators initiate the conversion of plasminogen to plasmin, which hydrolyzes polymerized fibrin strands into soluble fibrin degradation products. Infection may lead to an imbalance between platelet function and the regulatory mechanisms of the coagulation cascade and fibrinolysis, resulting in bleeding, thrombosis, or both.

3.2.1. Antithrombin

Antithrombin is the main inhibitor of thrombin and factor Xa. During severe infection, antithrombin levels are very low due to consumption, impaired synthesis primarily as a result of endothelial dysfunction and degradation by elastase from activated neutrophils [47,48]. A reduced antithrombin function is further mediated by a decreased availability of glycosaminoglycan at the perturbed endothelial surface. Under normal conditions, glycosaminoglycans act as physiological heparin-like co-factors that catalyze antithrombin [49]. Decreased concentration of glycosaminoglycans inactivates less thrombin, Xa and IXa. This results in a procoagulant state.

3.2.2. Protein C

The involvement of endothelial dysfunction in the impaired functioning of the protein C system is even more apparent. Under physiological conditions, protein C is activated by thrombin bound to thrombomodulin [50]. This complex together with protein S cleaves factors Va and VIIIa. So thrombomodulin and protein S generate a negative feedback via protein C to the generation of thrombin [51]. In cell cultures proinflammatory agents, such as tumor necrosis factor (TNF)-α and IL-1, reduce the concentration of thrombomodulin [52]. As a result, they compromise thromboresistance to factors Va and VIIIa. In sepsis, in addition to the already low levels of protein C, the main cause of protein C system dysfunction is this down-regulation of thrombomodulin [53]. Furthermore, endothelial cells, primarily from large blood vessels, express an endothelial protein C receptor (EPCR) that enhances the activation of protein C on the cell surface [54]. During sepsis this EPCR is reduced, causing further impairment of the protein C anticoagulation pathway [55].

3.2.3. Tissue factor pathway inhibitor

A third inhibitory mechanism of thrombin generation involves tissue factor pathway inhibitor (TFPI), which is synthesized and stored in endothelial cells. This molecule inhibits TF-factor VIIa complex by forming a quaternary complex in which factor Xa is the fourth component. The relevance of TFPI in the coagulopathy of severe inflammation is demonstrated in several experimental studies [56,57]. A recent study in healthy human volunteers confirmed the potential of TFPI to block the procoagulant pathway triggered by endotoxines [58]. However, clinical studies in septic patients have not provided clues to its clinical importance, because in the majority of the patients...
the levels of TFPI are not diminished compared with normal subjects [59].

3.2.4. Fibrinolysis

Endothelial cells are involved in the fibrinolytic process by release of urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA) and its modulator tissue plasminogen activator inhibitor 1 (PAI-1). Fibrinolysis is activated during severe infections, demonstrated in an experimental setting [7], mainly by activation of endothelial cells by cytokines [60] and in response to fibrin formation as well. Fibrinolysis, triggered by coagulation activation in severe inflammation [61], is shut off rapidly by the release of relatively large amounts of PAI-1. After infection of TNF-α and lipopolysaccharide (LPS) in healthy volunteers, activation of the coagulation cascade is preceded by a transient activation of fibrinolysis, which is reflected by increased levels of circulating tPA and urokinase plasminogen activator followed by an increase in PAI-1 [62–64]. The elevated levels of PAI-1 depress the fibrinolytic system, resulting in a procoagulant state [7].

3.3. Interaction between coagulation and inflammation: the crosstalk

The impairment of the endothelial anticoagulant mechanism by proteases that affect the inflammatory response follows similar pathways to those of coagulation proteases. Several endothelial derived mediators of blood coagulation have a major impact on the inflammatory host response [65]. Factor Xa, thrombin and factor VIIa–TF complex have each been shown to elicit pro-inflammatory activities [66,67]. Thrombin stimulated endothelial cells express IL-6, IL-8 and monocyte chemotactic protein-1 (MCP-1) [68]. Exposure of cultured endothelial cells to factor Xa stimulated the production of MCP-1, IL-6 and IL-8, and increased expression of adhesion molecules that mediated adhesion of leukocytes [69]. Furthermore, the coagulation system of endothelial cells is an important feedback activator of systemic infection. The in vivo anti-inflammatory properties of activated protein C and antithrombin are well known [67,70,71].

3.3.1. Cytokines

During sepsis, endothelial cells are the target organs for bacteria, and viruses and cytokines. On the endothelial surface, these cytokines regulate the expression of several proteins involved in coagulation and fibrinolysis [62]. The derangement of coagulation and fibrinolysis is mediated by IL-6 and IL-8 [72]. Cytokines such as, TNF-α induces the expression of thrombomodulin on endothelial cells [73] and influences indirectly the activation of coagulation because of its effects on IL-6. Furthermore IL-6 is the pivotal mediator of the deregulation of endothelial anti-coagulant pathways and the fibrinolytic defect [72]. In vivo studies have shown that treatment of primates with anti IL-6 after the administration of low-dose endotoxin prevents activation of the coagulation cascade but does not affect fibrinolysis [74]. The administration of anti IL-1 results in inhibition of both the coagulation cascade and fibrinolysis in baboons with lethal bacteraemia and patients with the sepsis syndrome [75,76]. Anti-inflammatory cytokines, such as IL-10, may modulate the activation of coagulation: administration of recombinant IL-10 to humans completely eliminated endotoxin-induced effects on coagulation [77]. Taken together, a number of coagulation proteases are able to induce pro-inflammatory mediators of endothelial cells that again have procoagulant effects. In particular vascular endothelial cells seem to play a pivotal mediatory role in the coagulation response to systemic inflammation and the interaction between coagulation and inflammation [78].

3.4. Soluble adhesion molecules

In the endothelial process of transformation into a procoagulant state, surface adhesion molecules are of major importance [79]. Endothelial cells express several adhesion molecules on their surface, including E-selectin (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1) and P-selectin, presumably in response to stimulation with cytokines or thrombin [80]. Soluble ICAM-1, VCAM-1 and platelet endothelial cell adhesion molecules-1 (PECAM-1) play an essential role in the binding of leukocytes [81], since P-selectin [82] and E-selectin are expressed by endothelial cells [83] and function as leukocytes-binding elements (Fig. 2). The subsequent influx of leukocytes results in a local inflammatory response, endothelial damage, and plasma leakage and shock [8]. Blockage of these adhesion molecules with antibodies improves sepsis-associated organ dysfunction [84].

During shock and cardiovascular disease elevated levels of adhesion molecules could be a sign of endothelial damage. In patients with septic shock elevated levels of adhesion molecules are found [85]. In peripheral artery disease and coronary artery disease, the levels of ICAM-1 are elevated. These levels could be predictors of thrombotic disorders [86] and ischemic heart disease [87], but results are still inconclusive. The levels of sVCAM-1 and sPECAM-1 do not predict an adverse outcome in these diseases [88]. Still the finding of increased plasma concentrations of these endothelial surface adhesion molecules is thought to reflect the level of activation and perhaps damage of the endothelial cell [89,90]. In the clinical setting, measurement of E-selectin may be of diagnostic or prognostic value for diseases associated with endothelial pathology, since it is expressed only by vascular endothelial cells and not by any other cell types [91].
3.5. Circulating endothelial cells

Activated endothelial cells, thrombocytes and monocytes release small particles from their outer membrane [92–94]. These so-called microparticles (MP) expose phospholipids, thereby providing binding sites for activated coagulating factors [95]. In vitro experiments have shown that after activation with LPS, endothelial cells have fewer intercellular adherent junctions [96] and that endothelial-derived MP may have procoagulant activity [97]. It has been demonstrated in vivo, that endotoxin increases the number of circulating endothelial cells by injury of the endothelial layer and detachment of endothelial cells from the basal membrane [98].

Only in patient with severe sickle cell disease, MP derived from endothelial cells are a source of TF [99]. Further evidence of the role of endothelial MP cells during inflammation is still not present. Thrombocytes and monocytes derived MP with a procoagulant activity are found in the blood during severe meningococcal sepsis [100]. And recently it was shown that in the circulation of patients with systemic lupus erythematosus (SLE) elevated levels of MP are found [101]. Measuring MP by immunofluorescence or immunomagnetic separation may provide additional information about activity of vascular endothelium [99].

4. Conclusion

Endothelium plays a key role in the pathogenesis of coagulation disorders in infectious diseases, although the precise mechanisms are not yet always clear. The endothelium is involved in both bacterial and non bacterial infections and is important for the initiation and regulation of haemostasis. Infection and/or activation of endothelial cells lead to acute complications such as thrombosis, haemorrhage and DIC, when chronic it may result in atherosclerosis or vasculitis. Although direct interactions between the infectious agent and the endothelial cells occur, cytokines—also endothelial-derived—are believed to be important mediators in this process.

During systemic gram-negative and gram-positive bacterial infections, activation of coagulation is mediated via the extrinsic TF pathway. Experimental studies suggest that, as a rule, coagulation and fibrinolysis occur independently of one another, and the overall result is usually a procoagulant state. The latter may result in DIC with microvascular thrombosis and organ failure. Bleeding in infectious diseases is probably a multifactor process resulting from a combination of endothelial damage or vascular leakage, thrombocytopenia, consumption of clotting factors and hyperfibrinolysis. In addition, immunologically mediated vasculitis may contribute to bleeding in...
specific infections. Crosstalk between coagulation and infection pathways is of major importance. The regulatory mechanisms of endothelial cells such as antithrombin, protein C, TFPI and the fibrinolytic system have been studied in detail [102]. Endothelial cells are involved in all pathways leading to haemostatic abnormalities.

Major emphasis is put on the interaction between inflammation and coagulation. Knowledge of the underlying mechanisms leading to thrombosis or bleeding is fundamental for the development of therapeutic strategies. Given the potential role that endothelial injury plays in some of the thrombohaemorrhagic complications of inflammation, we hypothesize that intervention in the coagulation pathways may favourably alter the clinical course of these infections. Many issues remain to be answered, and thus there is an urgent need for more studies, both clinical and experimental. Clinical studies must provide the answers to the question which infections are susceptible to disturbed haemostatic balance, and more precisely whether there is a disturbance of the coagulation pathway, the fibrinolytic cascade or both. Experimental studies have to provide insights into the mechanisms of interaction between different pathogens and endothelial cells, i.e. which coagulation proteins and which cytokines are involved. Which pathogens have a tendency to initiate a chronic inflammatory reaction which may result in chronic complications like vasculitis and/or atherosclerosis. A better understanding may open the way to new therapeutic modalities such as vaccination and antibiotic regimens. It is time to start clinical intervention trials to prevent the prothrombotic state.

References


