The normal role of Activated Protein C in maintaining homeostasis and its relevance to critical illness

Charles T Esmon

Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Howard Hughes Medical Institute, Oklahoma City, Oklahoma, USA

Correspondence: Dr C T Esmon, Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Howard Hughes Medical Institute, 825 NE 13th Street, Oklahoma City, OK 73104, USA. Tel: +1 405 271 6474; fax: +1 405 271 3137; e-mail: Charles-Esmon@omrf.ouhsc.edu

Abstract

Thrombin is a multifunctional protein, with procoagulant, inflammatory and anticoagulant effects. Binding of thrombin to thrombomodulin results in activation of Protein C and initiation of the Activated Protein C anticoagulant pathway, a process that is augmented by the endothelial cell Protein C receptor (EPCR). Activated Protein C has demonstrated antithrombotic, anti-inflammatory, and profibrinolytic properties. Its antithrombotic activity is particularly important in the microcirculation, and Protein C deficiency is associated with microvascular thrombosis. Activated Protein C has also been shown to modulate inflammation. When the level of thrombomodulin or Protein C is reduced in sepsis there is a vicious cycle of coagulation and inflammation, with potentially lethal consequences. *In vitro* studies and animal models have shown that Activated Protein C blunts the inflammatory and coagulant response to sepsis through a variety of mechanisms.

Keywords: Activated Protein C, coagulation, endothelial cell Protein C receptor (EPCR), inflammation, thrombomodulin
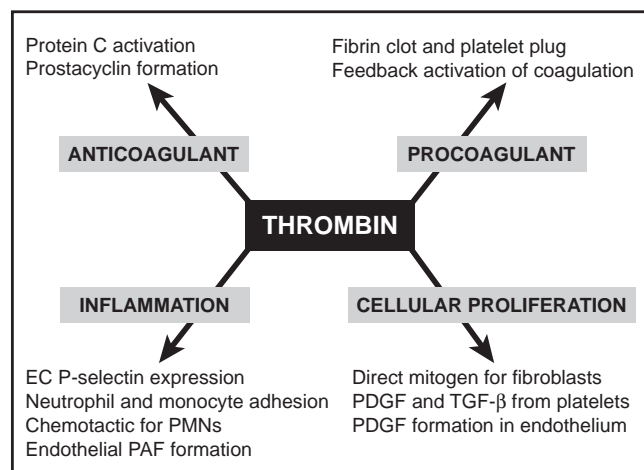
Introduction

The initiation of coagulation, especially in severe sepsis, is probably mediated by the induction of tissue factor (TF) expression by endotoxin. This leads to the activation of factor X, which then combines with factor Va to convert prothrombin to thrombin. Although thrombin is usually considered to have purely procoagulant activity, it is in fact a multifunctional protein that has some important homeostatic anticoagulant effects (Fig. 1) [1]. One of its main functions is to bind to thrombomodulin, which is expressed on the endothelial cell surface. Thrombomodulin is the major physiologic buffer for the procoagulant effects of thrombin in normal vessels [2,3]. Because thrombomodulin binds to the same site on thrombin that would normally bind to fibrinogen, platelets or factor V, all

of these functions are blocked. Instead, the thrombin–thrombomodulin complex activates Protein C (through a different site on the thrombin molecule), resulting in initiation of the Activated Protein C pathway [3]. This process is augmented by the EPCR [4,5]. Activated Protein C must dissociate from EPCR before it can bind to Protein S and function as an effective anticoagulant through the inactivation of factor Va.

Thrombomodulin and Activated Protein C in the microcirculation

Activated Protein C is particularly important in the microcirculation [3]. Although the number of thrombomodulin molecules per endothelial cell is approximately constant, the local concentration of thrombomodulin is determined

---

C4BP = C4-binding protein; EPCR = endothelial cell Protein C receptor; PAI = plasminogen activator inhibitor; TF = tissue factor; TNF = tumor necrosis factor.

Figure 1

Cellular and humoral responses to nM concentrations of thrombin. EC, endothelial cell; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PMN, polymorphonuclear leukocyte; TGF, transforming growth factor. Adapted from Esmon [1].

Figure 2

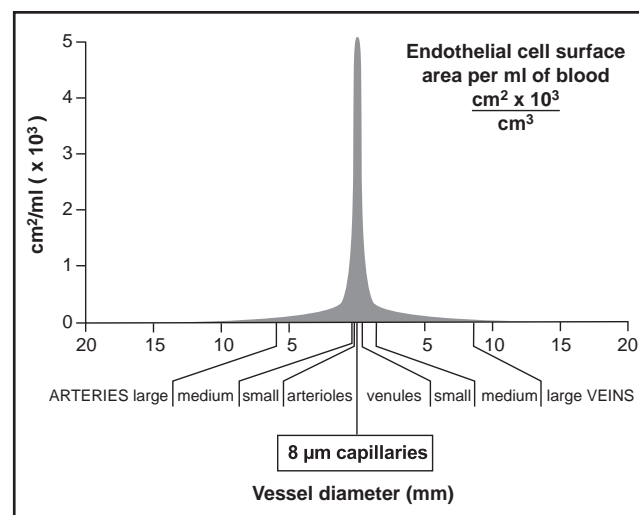Endothelial cell surface area per ml of blood. Adapted from Busch *et al* [6].

by the number of endothelial cells that are in contact with the blood. Because the endothelial cell surface area per unit blood volume is much greater in the microcirculation (approximately 3000–5000 cm^2 of endothelium/ml blood) than in larger blood vessels (approximately 1.5 cm^2/ml) (Fig. 2) [6], there is a correspondingly high concentration of thrombomodulin in the normal microvasculature (approximately 500 nmol/l) as compared with the larger vessels (approximately 0.1–0.2 nmol/l). As a result, thrombin is rapidly removed from the microcirculation as it is bound to thrombomodulin. This suggests that the Activated Protein C system is uniquely poised to regulate coagulopathies in the microcirculation, and this has been confirmed in clinical studies [7].

Importantly, at least in some forms of sepsis, thrombomodulin can be downregulated, with resulting loss of ability to regulate thrombin, and hence clotting, in the microcirculation. Homozygous Protein C deficiency has a similar result, such that affected babies develop microvascular thrombosis (purpura fulminans) almost immediately after birth [3,8]. In contrast, antithrombin deficiencies cause large-vessel thrombosis.

Vicious cycle of coagulation and inflammation

In addition to its procoagulant and anticoagulant effects, thrombin can also be involved in the process of inflammation (Fig. 1) [1]. That is, it can activate endothelial cells to express P-selectin, causing neutrophil and monocyte adhesion. It is also chemotactic for polymorphonuclear leukocytes and is a potent inducer of endothelial platelet-activating factor formation. Platelet-activating factor is a major activator of neutrophils, which have

been demonstrated to play an important role in the pathogenesis of sepsis.

The inflammatory response also modulates the coagulation system, including downregulation of the expression of thrombomodulin (Fig. 3) [9]. Inflammation also increases levels of α_1-antitrypsin, which is an acute-phase response protein and an important inhibitor of the Protein C pathway. Acute inflammatory mediators such as tumor necrosis factor (TNF)-α and endotoxin amplify the production of TF, particularly by monocytes, triggering further coagulation. Complement activation by endotoxin increases exposure of clot-promoting membrane phospholipids to blood, and thereby propagates the coagulation response. In certain forms of inflammation, levels of both fibrinogen and plasminogen activator inhibitor (PAI)-1 (an inhibitor of fibrinolysis) are increased.

If there were no mechanisms to disrupt the amplification of coagulation by inflammation, and *vice versa*, then the ensuing vicious cycle would have a catastrophic effect, leading ultimately to death (Fig. 4) [10]. It was hypothesized, therefore, that endogenous Activated Protein C interferes with these amplification loops by modulating both coagulation and inflammation, which differentiates it from other types of anticoagulants, and that Protein C depletion may occur in severe sepsis [11].

A primate model was used in the initial study undertaken to investigate this possibility [11]. In animals infused with a lethal *Escherichia coli* dose alone, Protein C and fibrinogen levels fell, liver damage occurred (Fig. 5a), and the animals died within 24–32 h. In contrast, organ damage
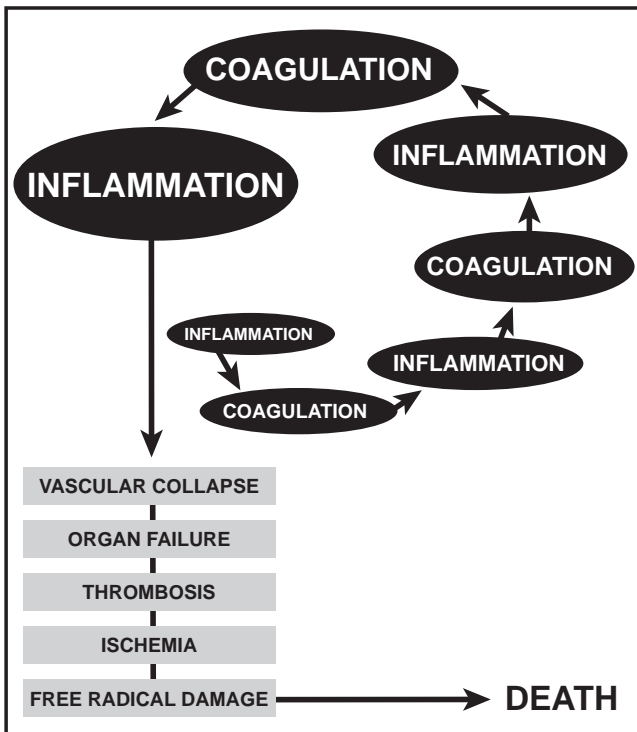
was prevented in animals given a lethal dose of *E. coli* plus an infusion of Activated Protein C (Fig. 5b). Importantly, it was shown that animals could still be rescued if

Figure 3
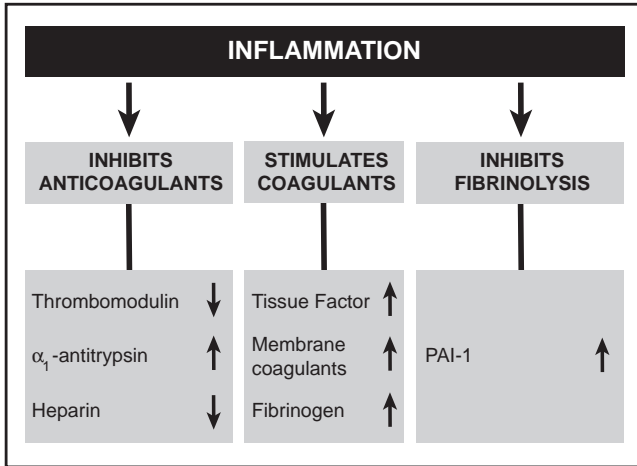
Roles of inflammatory mediators in the coagulation response. Adapted from Esmon [9].
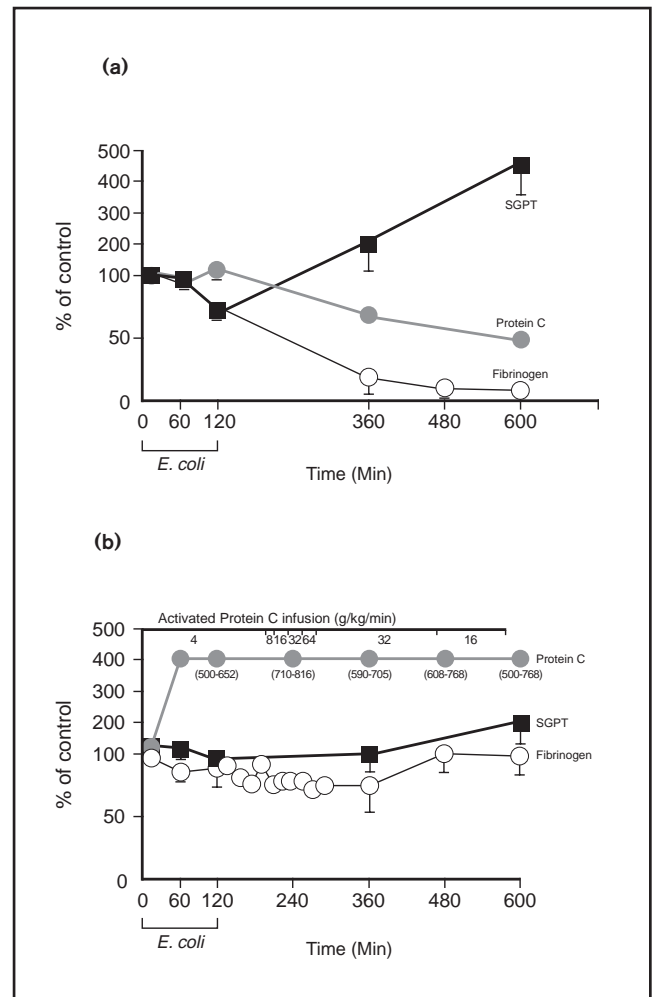
Figure 4

The inflammation–coagulation autoamplification loop. Adapted from Esmon *et al* [10].

Activated Protein C was given 2–3 h after the lethal *E. coli* dose was administered. Similarly, when animals were given a sublethal dose of *E. coli* and Protein C activation was blocked with an antibody, mimicking the clinical situation in a patient with sepsis and low Protein C levels, the sublethal bacterial dose became lethal, with complete fibrinogen consumption, a disseminated intravascular coagulation-like syndrome, organ failure, and a full-blown septic shock-like response (Fig. 6) [11].

Amplification of the inflammatory mediators in this setting aggravates the process still further. C4-binding protein (C4BP) is an acute-phase protein that is known to bind and inhibit Protein S, a vital cofactor for the Activated Protein C system. In another primate study [12], it was shown that administration of C4BP together with a

Figure 5

Primate model of sepsis. **(a)** Lethal *E. coli* infusion alone. **(b)** Lethal *E. coli* plus Activated Protein C infusion. SGPT, serum glutamic pyruvic transaminase (indicator of liver damage). Adapted from Taylor *et al* [11].
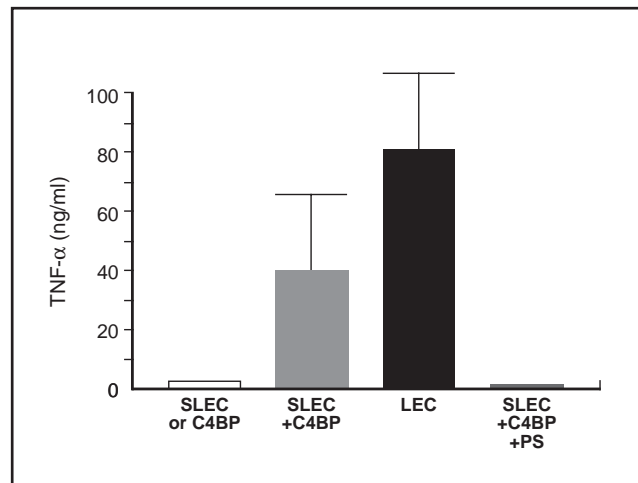
Figure 6
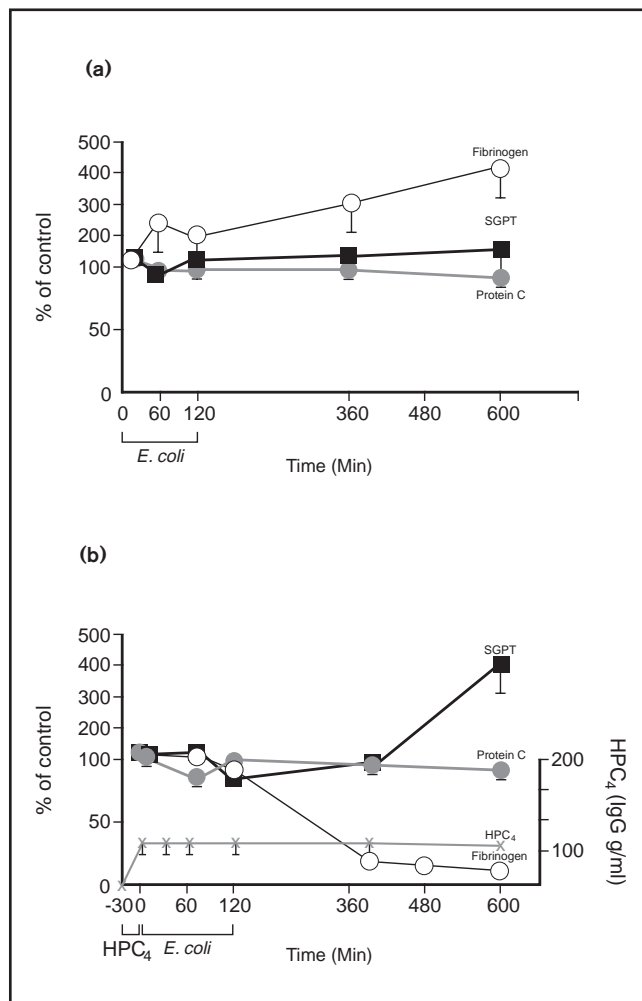
Primate model of sepsis. **(a)** Sublethal *E. coli* infusion alone.
(b) Sublethal *E. coli* plus antiProtein C monoclonal antibody infusion.
HPC$_4$, murine monoclonal antibody to human Protein C; Ig,
immunoglobulin; SGPT, serum glutamic pyruvic transaminase (indicator
of liver damage). Adapted from Taylor *et al* [11].

Figure 7

TNF-α production in response to sublethal or lethal *E. coli* infusion,
with or without C4BP and Protein S (PS) supplementation. C4BP and
PS were administered at doses of 20 mg/kg and 3 mg/kg,
respectively. SLEC, 10% of a lethal dose of *E. coli*; LEC, 100% lethal
dose dose of *E. coli*. Adapted from Taylor *et al* [12].

normally sublethal dose of *E. coli* resulted in complete
fibrinogen consumption and death. In the same study, it
was shown that, whereas either sublethal *E. coli* doses or
C4BP alone had no appreciable effect on TNF-α produc-
tion, administration of both substances led to a circulating
TNF-α level that approached that seen with a lethal dose
of *E. coli* (Fig. 7), with rapid consumption of fibrinogen,
systemic organ damage, and eventual death. With co-
administration of Protein S, however, TNF-α levels
remained similar to those seen in animals administered a
sublethal dose of *E. coli* alone (Fig. 7), and all of these
animals survived. This suggests that reversal of the
inhibitory effects of inflammatory cytokines on the Acti-
vated Protein C pathway can control the inflammatory
response.

Activated Protein C can promote fibrinolysis by at least
two mechanisms. One involves the ability of Activated
Protein C to form a tight complex with PAI-1. Once the
complex with Activated Protein C forms, PAI-1 (the major
inhibitor of tissue plasminogen activator) can no longer
inhibit tissue plasminogen activator [13]. Because of the
ability of Activated Protein C to limit thrombin generation,
it can also reduce the activation of a precarboxypeptidase
B commonly referred to as thrombin activatable fibrinolysis
inhibitor [14,15]. Thrombin activatable fibrinolysis inhibitor
functions by removing lysine residues from the fibrin clot
that would normally play a role in augmenting plasminogen
activation and the fibrinolytic activity of plasmin.

The potentially protective effect of Activated Protein C has
also been shown in two rat studies [16,17], both of which
suggested inhibition of leukocyte activation as an impor-
tant mechanism. In one of these studies [16], Activated
Protein C was shown to reduce motor disturbance result-
ing from spinal cord injury by reducing local levels of
TNF-α, thereby inhibiting neutrophil accumulation. In the
other study [17], animals treated with Activated Protein C
were found to have a reduction in ischemia/reperfusion-
induced renal injury, which was associated with reductions
in renal levels of TNF-α, interleukin-8 and myeloperoxidase,
with resulting inhibition of leukocyte activation.

Unique properties of the Activated Protein C pathway
Activated Protein C has a number of properties that differ-
entiate it from other anticoagulants, such as heparin or

Figure 8
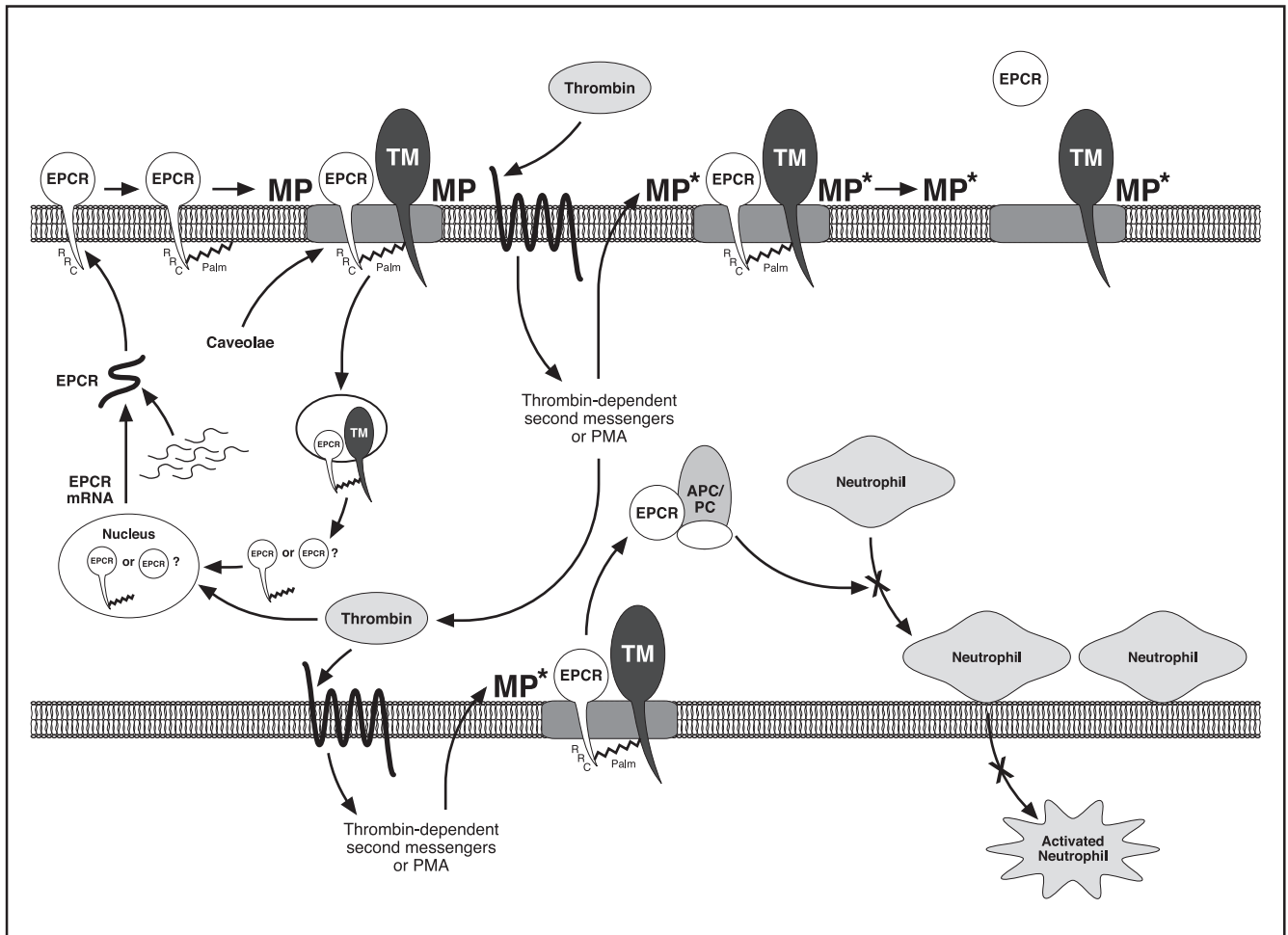
Activated Protein C/EPCR-mediated anticoagulant pathway. APC, Activated Protein C; MP, metalloproteinase; Palm, palmitate; PC, Protein C; PMA, phorbol myristate acetate; TM, thrombomodulin; RRC, Arg,Arg,Lys. Adapted from Esmon [18].

antithrombin. First, the Activated Protein C system is geared to modulate coagulopathy within the microvasculature, and it is microvascular disease that lies at the root of sepsis. Second, the Activated Protein C system involves the EPCR, which was identified during research to discover possible receptors and pathways linked to the system [17]. The EPCR was found to be homologous to the major histocompatibility complex class I family of molecules [4], all of which are involved in the regulation of inflammation.

The Activated Protein C/EPCR-mediated anticoagulant pathway is illustrated in Fig. 8 [18]. The EPCR is synthesized on the endothelial cell surface and moves to specialized regions (caveolae) in the endothelium, through which it is able to enter the cell and then the cell nucleus. Once inside the nucleus, it is able to redirect the gene expression profiles within the cell. Unlike other plasma membrane

receptors, EPCR is also capable of carrying Activated Protein C into the nucleus, with subsequent alterations to some of the gene products.

Furthermore, when thrombin or inflammatory cytokines come into contact with the endothelium, they induce shedding of EPCR from the cell surface, a process that is mediated by a metalloproteinase [5]. Soluble EPCR binds to proteinase 3, a serine proteinase that is found in neutrophils. This soluble EPCR–proteinase 3 complex, either alone or in a complex with Protein C, appears to interact with neutrophils via an integrin (Mac-1) to inhibit their adhesion to the endothelium, and as a result prevents the migration of the neutrophils into the tissues [19].

Several unique mechanisms by which Activated Protein C can affect inflammation and coagulation have been demonstrated in various *in vitro* studies [20–23]. In one study [20],

it was shown that incubation of monocytes with Activated Protein C leads to a decrease in TNF-α production and blockade of the translocation and activation of the central proinflammatory transcription factor nuclear factor-κB in response to lipopolysaccharide exposure. The binding of Activated Protein C to a specific receptor on mononuclear phagocytes has also been shown to inhibit intracellular calcium signaling and monocyte-dependent T-cell proliferative responses [21]. Finally, EPCR-dependent TF suppression by Activated Protein C has been demonstrated in a human monoblastic leukemia cell line [22].

Conclusion

The sepsis cascade, which is associated with simultaneous activation of systemic inflammation and coagulation, along with altered fibrinolysis, leads to microvascular endothelial injury, acute organ dysfunction, and possibly death. Thrombin, with its procoagulant and anticoagulant effects, as well as its involvement in the process of inflammation, is thought to be central to the sepsis cascade. *In vitro* and animal models suggest that Activated Protein C, a protein that has antithrombotic, anti-inflammatory and profibrinolytic activities, may be an important modulator of the vicious cycle of coagulation and inflammation associated with the sepsis cascade, which if unchecked may ultimately lead to death. Several properties of Activated Protein C, including its unique positioning to regulate coagulopathies in the microvasculature and the ability of EPCR to facilitate Protein C activation, support the continued development of Activated Protein C-mediated strategies that are aimed at disrupting the process whereby inflammation initiates coagulation and coagulation amplifies inflammation.

References

1. Esmon C: **Cell mediated events that control blood coagulation and vascular injury.** *Ann Rev Cell Devel Biol* 1993, **9**:1–26.
2. Waugh JM, Li-Hawkins J, Yuksel E, Kuo MD, Cifra PN, Hilfiker PR, Geske R, Chawla M, Thomas J, Shenaq SM, Dake MD, Woo SLC: **Thrombomodulin overexpression to limit neointima formation.** *Circulation* 2000, **102**:332–337.
3. Esmon CT: **Natural anticoagulants and their pathways.** In: *Antithrombotics (Handbook of Experiemenal Pharmacology*, vol 132. Edited by Uprichard ACG, Gallagher KP. Heidelberg: Springer-Verlag; 1999:447–476.
4. Fukudome K, Esmon CT: **Identification, cloning and regulation of a novel endothelial cell protein C/activated protein C receptor.** *J Biol Chem* 1994, **269**:26486–26491.
5. Esmon CT: **The endothelial cell protein C receptor.** *Thromb Haemost* 2000, **83**:639–643.
6. Busch C, Cancilla P, DeBault L, Goldsmith J, Owen W: **Use of endothelium cultured on microcarriers as a model for the microcirculation.** *Lab Invest* 1982, **47**:498–504..
7. Fuentes-Prior P, Iwanaga Y, Huber R, Pagila R, Rumennik G, Seto M, Morser J, Light DR, Bode W: **Structural basis for the anticoagulant activity of the thrombin–thrombomodulin complex.** *Nature* 2000, **404**:518–525.
8. Smith OP, White B: **Infectious purpura fulminans: diagnosis and treatment.** *Br J Haematol* 1999, **104**:202–207.
9. Esmon CT: **Inflammation and thrombosis: mutual regulation by protein C.** *The Immunologist* 1998, **6**:84–89.
10. Esmon CT, Fukudome K, Mather T, Bode W, Esmon NL, Regan LM, Stearns-Kurosawa DJ, Kurosawa S: **Inflammation: the protein C pathway.** *Fibrinolysis Proteolysis* 1997, **11**:143–148.
11. Taylor FB Jr, Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE: **Protein C prevents the coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon.** *J Clin Invest* 1987, **79**:918–925.
12. Taylor F, Chang A, Ferrell G, Mather T, Catlett R, Blick K, Esmon CT: **C4b-binding protein exacerbates the host response to *Escherichia coli*.** *Blood* 1991, **78**:357–363.
13. Sakata Y, Curriden S, Lawrence D, Griffin JH, Loskutoff DJ: **Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity.** *Proc Natl Acad Sci USA* 1985, **82**:1121–1125.
14. Bajzar L, Morser J, Nesheim M: **TAFI or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin–thrombomodulin complex.** *J Biol Chem* 1996, **271**:16603–16608.
15. Bajzar L, Manuel R, Nesheim ME: **Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor.** *J Biol Chem* 1995, **270**:14477–14484.
16. Taoka Y, Okajima K, Uchiba M, Murakami K, Harada N, Johno M, Naruo M: **Activated protein C reduces the severity of compression-induced spinal cord injury in rats by inhibiting activation of leukocytes.** *J Neurosci* 1998, **18**:1393–1398.
17. Mizutani A, Okajima K, Uchiba M, Noguchi T: **Activated protein C reduces ischemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation.** *Blood* 2000, **95**:3781–3787.
18. Esmon CT: *Activated Protein C.* London: ReMedica Publishing; 2001:in press.
19. Kurosawa S, Esmon CT, Stearns-Kurosawa DJ: **The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18.** *J Immunol* 2000, **165**:4697–4703.
20. White B, Schmidt M, Murphy C, Livingstone W, O'Toole D, Lawler M, O'Neill L, Kelleher D, Schwarz HP, Smith OP: **Activated protein C inhibits lipopolysaccharide-induced nuclear translocation of nuclear factor κB (NF-κB) and tumour necrosis factor α (TNF-α) production in the THP-1 monocytic cell line.** *Br J Haematol* 2000, **110**:130–134.
21. Hancock WW, Grey ST, Hau L, Akalin E, Orthner C, Sayegh MH, Salem HH: **Binding of activated protein C to a specific receptor on human mononuclear phagocytes inhibits intracellular calcium signaling and monocyte-dependent proliferative responses.** *Transplantation* 1995, **60**:1525–1532.
22. Shu F, Kobayashi H, Fukudome K, Tsuneyoshi N, Kimoto M, Terao T: **Activated protein C suppresses tissue factor expression on U937 cells in the endothelial protein C receptor-dependent manner.** *FEBS Lett* 2000, **477**:208–212.
23. Taylor FB Jr, Stearns-Kurosawa DJ, Kurosawa S, Ferrell G, Chang ACK, Laszik Z, Kosanke S, Peer G, Esmon CT: **The endothelial cell protein C receptor aids in host defence against *Escherichia coli* sepsis.** *Blood* 2000, **95**:1680–1686.