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Inflammation-associated activation of coagulation and immune regulation by the protein C pathway

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Abstract

The inflammation-induced activation of the protein C pathway provides negative feedback inhibition of coagulation and exerts coagulation-independent anti-inflammatory and cytoprotective effects. The balance between these activities of aPC modulates the outcome of diverse inflammatory diseases such as encephalitis, diabetes, and sepsis; and is affected by naturally occurring aPC-resistance of coagulation factor V Leiden.

Keywords

Inflammation; Protein C; factor V Leiden

INTRODUCTION

The activation of protein C by the Thrombomodulin-thrombin complex provides effective feedback inhibition of Tissue Factor-initiated coagulation. In addition to its anticoagulant activity, aPC exerts a wide spectrum of cytoprotective and anti-inflammatory effects in endothelial cell and immune cells that are mediated by a number of signaling-competent cell surface receptors [1, 2]. The Arg506Gln mutation in human fV *Leiden* exerts its prothrombotic effect by disrupting the anticoagulant activity of aPC towards activated factors Va and VIIIa [3]. On the other hand, provided a sufficient bioavailability of Thrombomodulin and protein C, enhanced thrombin formation also leads to increased activation of protein C and thereby might promote coagulation-independent functions of aPC. The coupling of thrombin generation to aPC formation also raises questions about the effect of anticoagulation therapy on coagulation-independent anti-inflammatory and cytoprotective functions of the protein C pathway. This complex interdependence of coagulation activation and protective protein C pathway functions is highlighted by the

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Conflict of Interest

The author has no conflicts of interest to disclose

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outcomes of recent studies addressing the role of aPC in murine models of encephalitis, diabetes and diabetic nephropathy, and infectious disease.

APC effects on Experimental Autoimmune Encephalitis

Two studies have addressed the potential role of aPC in experimental models of autoimmune encephalitis (EAE). Strikingly, these studies show that inhibition of endogenous aPC and pharmacologic supplementation of recombinant aPC both ameliorate inflammatory CNS disease. Han et al. [4], employing proteomic profiling of in vivo lesions from MS patients, found that Tissue Factor and protein C inhibitor (PCI) are two proteins that are specifically associated with chronic active plaques. They then show in an EAE model of immunization with the glial cell antigen-derived peptide (proteolipid protein, PLP 139–151) that daily administration of the thrombin inhibitor hirudin, with treatment initiated at the peak of overt disease (complete hind limb paralysis), restored motor function for up to two weeks after treatment was begun. Thereafter, EAE relapsed, possibly due to the development of hirudin-neutralizing antibodies, indicating that hirudin therapy did not induce tolerance to the PLP antigen. The beneficial effects of thrombin-inhibition were associated with reduced in vitro lymphocyte reactivity towards re-stimulation with PLP peptide, and reduced in vivo abundance of inflammatory foci in the brain and spinal cord. Daily administration of recombinant murine aPC largely replicated the effects of hirudin. Two human aPC variants with diminished ability to interact with EPCR (L8V-aPC) and thus lacking the ability to engage EPCR-dependent cytoprotective signaling pathways, or reduced anticoagulant activity (K139E-aPC) both improved disease severity, leading the authors to conclude that the signaling and the anticoagulant function of aPC both contribute to its therapeutic benefits. The relevant in vivo cellular and molecular targets of aPC or thrombin were not further identified in this study. The notion that inhibition of endogenous aPC worsens EAE pathology was indeed tested in a more recent study by Alabanza et al. [5] employing PC/aPC-neutralizing antibodies. Similar to the work by Han et al., EAE was induced by immunization with a peptide derived from a glial cell antigen (myelin oligodendrocyte glycoprotein; MOG35-55). Infusion of antibodies that neutralize both aPC and PC during the induction phase of EAE (i.e. day 0 through day 6 after immunization) significantly delayed the onset of disease and moderately reduced disease severity. PC/aPC inhibition was associated with increased abundance of regulatory T-cells and IL-10 production in the brain, reduced leukocyte infiltration in the spinal cord, and reduced microglial activation, but also caused significantly increased blood-brain barrier permeability. Analysis of various lymphocyte and myeloid cell population on the spleen showed that PC/aPC inhibition elicited an increase in the number of myeloid CD11b^{POS} cells that coexpressed the Ly6C antigen, and by this criterion resembled so-called myeloid-derived suppressor cells (MDSC). MDSC from mice treated with PC-blocking antibodies indeed showed enhanced suppressive activity in re-stimulation splenocytes assays, and adoptive transfer of these cells almost completely suppressed EAE development. The authors proposed that endogenous aPC normally suppresses (via CD11b-Par1 signaling) the generation of specific MDSC subsets with immunosuppressive activity towards antigen-specific T-cells and the ability to elicit regulatory T cells, and that antibody-mediated PC/aPC inhibition would alleviate this inhibition. Given the importance of MDSC in trauma, sepsis, and cancer immune

surveillance, the proposed effects of aPC signaling on MDSC may help explain the puzzling multitude of inflammatory settings in which protein C pathway modulation alters disease outcome.

Thus it appears that positive or negative manipulation of aPC levels can paradoxically produce the same end result, i.e. amelioration of motor dysfunction. A notable difference between these studies is that aPC inhibition was achieved during the induction phase of EAE, and therefore may alter primarily the response to immunization with an auto-antigen, rather than countering the inflammatory sequelae of established disease, as in the study by Han.

APC-resistance of fV Leiden in Diabetes

Isermann et al. [6] reported that endogenous or therapeutically administered aPC improved glucose-mediated diabetic nephropathy in mice, and that this protective effect of aPC was mediated by activation of PAR1 in glomerular podocytes, which protected these cells from apoptosis. This protective effect of therapeutically administered aPC in diabetic pathologies was independently replicated in the NOD-mouse model of autoimmune type-1 diabetes [7]. In this model, aPC prevented spontaneous T1D onset, possibly due to enhanced generation of regulatory T cells. Of note, in the EAE model of induced autoimmune encephalitis, a similar expansion of regulatory was noted after *inhibition* of endogenous aPC[5]. Wang et al. [8] investigated the effect of aPC-resistant fV Leiden on diabetic nephropathy. Both heterozygous and homozygous Leiden mice showed reduced podocytes apoptosis and nephropathy, and the protective effect of fV Leiden was abolished by thrombin-inhibition with hirudin. Given that thrombomodulin-deficient mice show worsened nephropathy, a reasonable interpretation of these results is that thrombomodulin-mediated activation of endogenous protein C may be responsible for the protective effect of fV Leiden.

APC-resistance of fV Leiden in murine sepsis

The clinical use of recombinant aPC for the treatment of severe sepsis was based in part on the observation that infusion of thrombin prior to LPS challenge reduced mortality of endotoxic shock [9]. This prompted studies whether increased endogenous thrombin generation in mice with aPC resistance due to the fV Leiden mutation would similarly protect against endotoxemia. Heterozygous, but not homozygous Leiden animals were indeed protected, and a similar survival benefit was evident for heterozygous Leiden carriers enrolled in the placebo-arm of the PROWESS sepsis trial [10], but not in other clinical or pre-clinical studies of sepsis (for example [11, 12]). In a mouse model of pneumococcal pneumonia [13], fV Leiden had no lasting effect on inflammation, extent of coagulation, or survival if no antibiotic treatment was administered. However, if microbial load was controlled by antibiotic treatment, homozygous Leiden mice were protected from mortality. Remarkably, aside from reduced fXIII levels, the authors were unable to correlate the improved survival with corresponding alterations in biomarkers of coagulation, fibrinolysis, and inflammation, or with improved bacterial clearance. It is noteworthy that a similar lack of correlation between commonly used markers of systemic inflammation (such as plasma cytokine levels) and survival was also reported in mouse studies documenting the

therapeutic efficacy of recombinant aPC in endotoxemia and bacterial peritonitis [14, 15]. Such studies indicate that aPC-resistance of fV indeed modulates sepsis outcome, but also highlight that such effects are largely independent of this mutation's effect on the regulation of coagulation, and are likely based on as yet unknown mechanisms of endogenous aPC signaling.

Conclusion

The link between activation of the blood coagulation mechanism and inflammation has been rationalized by considering the coagulation reaction a specialized arm of the host defense against acute injury and infection that aids in containing pathogen invasion and spreading, and maintains integrity of vascular function. Emerging evidence suggests that the coagulation-dependent engagement of the endogenous protein C system modulates through as yet unknown molecular and cellular mechanism innate, as well as adaptive immune responses, rather than just exerting an overall “anti-inflammatory” effect. APC-resistance of fV Leiden modulates this balance between the anticoagulant and immune-regulatory functions of the protein C pathway and constitutes a naturally occurring paradigm for investigating how aPC modulates the outcomes of infectious and non-infectious inflammatory disease.

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References

- Griffin JH, Zlokovic BV, Mosnier LO. Protein C anticoagulant and cytoprotective pathways. *Int J Hematol.* 2012; 95:333–45. [PubMed: 22477541]
- Esmon CT. Protein C anticoagulant system--anti-inflammatory effects. *Sem Immunopath.* 2012; 34:127–32.
- Segers K, Dahlback B, Nicolaes GA. Coagulation factor V and thrombophilia: background and mechanisms. *Thromb Haemost.* 2007; 98:530–42. [PubMed: 17849041]
- Han MH, Hwang SI, Roy DB, Lundgren DH, Price JV, Ousman SS, et al. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature.* 2008; 451:1076–81. [PubMed: 18278032]
- Alabanza LM, Esmon NL, Esmon CT, Bynoe MS. Inhibition of endogenous activated protein C attenuates experimental autoimmune encephalomyelitis by inducing myeloid-derived suppressor cells. *J Immunol.* 2013; 191:3764–77. [PubMed: 23997223]
- Isermann B, Vinnikov IA, Madhusudhan T, Herzog S, Kashif M, Blautzik J, et al. Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat Med.* 2007; 13:1349–58. [PubMed: 17982464]
- Xue M, Dervish S, Harrison LC, Fulcher G, Jackson CJ. Activated protein C inhibits pancreatic islet inflammation, stimulates T regulatory cells, and prevents diabetes in non-obese diabetic (NOD) mice. *J Biol Chem.* 2012; 287:16356–64. [PubMed: 22447930]
- Wang H, Madhusudhan T, He T, Hummel B, Schmidt S, Vinnikov IA, et al. Low but sustained coagulation activation ameliorates glucose-induced podocyte apoptosis: protective effect of factor V Leiden in diabetic nephropathy. *Blood.* 2011; 117:5231–42. [PubMed: 21389321]
- Taylor FB Jr, Chang A, Hinshaw LB, Esmon CT, Archer LT, Beller BK. A model for thrombin protection against endotoxin. *Thromb Res.* 1984; 36:177–85. [PubMed: 6506035]

10. Kerlin BA, Yan SB, Isermann BH, Brandt JT, Sood R, Basson BR, et al. Survival advantage associated with heterozygous factor V Leiden mutation in patients with severe sepsis and in mouse endotoxemia. *Blood*. 2003; 102:3085–92. [PubMed: 12869495]
11. Benfield T, Ejrnaes K, Juul K, Ostergaard C, Helweg-Larsen J, Weis N, et al. Influence of Factor V Leiden on susceptibility to and outcome from critical illness: a genetic association study. *Crit Care*. 2010; 14:R28. [PubMed: 20202226]
12. Bruggemann LW, Schoenmakers SH, Groot AP, Reitsma PH, Spek CA. Role of the factor V Leiden mutation in septic peritonitis assessed in factor V Leiden transgenic mice. *Crit Care Med*. 2006; 34:2201–6. [PubMed: 16775574]
13. Schouten M, van't Veer C, Roelofs JJ, Levi M, van der Poll T. Impact of the factor V Leiden mutation on the outcome of pneumococcal pneumonia: a controlled laboratory study. *Crit Care*. 2010; 14:R145. [PubMed: 20682036]
14. Kerschen E, Hernandez I, Zogg M, Jia S, Hessner MJ, Fernandez JA, et al. Activated protein C targets CD8+ dendritic cells to reduce the mortality of endotoxemia in mice. *J Clin Invest*. 2010; 120:3167–78. [PubMed: 20714108]
15. Kerschen EJ, Fernandez JA, Cooley BC, Yang XV, Sood R, Mosnier LO, et al. Endotoxemia and sepsis mortality reduction by non-anticoagulant activated protein C. *J Exp Med*. 2007; 204:2439–48. [PubMed: 17893198]