

LETTER TO THE EDITOR

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Hypoxia and HIF activation as a possible link between sepsis and thrombosis



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Abstract

Risk factors for thrombosis include hypoxia and sepsis, but the mechanisms that control sepsis-induced thrombus formation are incompletely understood. A recent article published in *Thrombosis Journal*: (i) reviews the role of endothelial cells in the pathogenesis of sepsis-associated microthrombosis; (ii) describes a novel ‘two-path unifying theory’ of hemostatic disorders; and (iii) refers to hypoxia as a consequence of microthrombus formation in sepsis patients. The current article adds to this review by describing how sepsis and thrombus formation could be linked through hypoxia and activation of hypoxia-inducible transcription factors (HIFs). In other words, hypoxia and HIF activation may be a cause as well as a consequence of thrombosis in sepsis patients. While microthrombosis reduces microvascular blood flow causing local hypoxia and tissue ischemia, sepsis-induced increases in HIF1 activation could conversely increase the expression of coagulant factors and integrins that promote thrombus formation, and stimulate the formation of pro-thrombotic neutrophil extracellular traps. A better understanding of the role of cell-specific HIFs in thrombus formation could lead to the development of novel prophylactic therapies for individuals at risk of thrombosis.

Keywords: Endothelium, Hypoxia, Hypoxia-inducible factors, Integrins, Thrombosis

Dear Editor,

Thrombosis is a common condition with potentially debilitating and fatal consequences, but the mechanisms that control thrombus formation are incompletely understood. Risk factors for thrombosis include trauma, pregnancy, high altitude, and sepsis [1]. Deep vein thrombosis occurs in regions of low blood flow, potentially leading to pulmonary embolism and post-thrombotic syndrome. The incidence of venous thrombosis is approximately 1 in 500 per year in the general population [2]. Arterial thrombi form under conditions of higher turbulent flow and thromboembolism can lead to fatal myocardial infarction or stroke. The major treatment for thrombosis is anticoagulation, which prevents thrombus extension, but increases the risk of bleeding [3]. Other treatments include thrombolysis and thrombectomy, but these are contraindicated in many patients, and carry increased risks of excessive bleeding and re-thrombosis [3].

Furthermore, clinical trials of anti-coagulants in sepsis patients have failed [4, 5]. A better understanding of the mechanisms that control thrombus formation could lead to the development of improved prophylactic therapies for individuals at risk for thrombosis, including but not limited to sepsis patients.

Sepsis-induced organ injury is commonly associated with the formation of small vessel microthrombi and an article in *Thrombosis Journal* has carefully reviewed the molecular mechanisms that control sepsis-induced microthrombosis with a focus on the endothelial cell response [5]. This recently-published review refers to hypoxia as a consequence of microthrombosis in sepsis [5], but it is also possible that hypoxia triggers microthrombus formation in sepsis patients [6, 7]. The review by Chang also refers to another article by the same author that describes the pathogenesis of disseminated intravascular microthrombosis and introduces a ‘two-path unifying theory’ of haemostatic disorders [8]. According to this theory, “normal” hemostasis is triggered by simultaneous but independent activation of tissue factor (TF) and “unusually large von Willebrand factor multimers”, while sepsis-associated endotheliopathy is triggered by

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activation of the unusually large von Willebrand factor multimers alone [5, 8]. In the more recent review article [5], Chang states that “DIC [disseminated intravascular coagulation] has been inappropriately conceptualized as a fibrin clot disease produced via activated TF/FVIIa-initiated cascade/cell-based coagulation” and that “consumption coagulopathy in acute promyelocytic leukaemia that occurs due to pathologic activation of aberrant TF path caused by TF released from leukemic promyelocytes should be called true DIC [8]”. The author also interestingly writes that “True DIC in acute promyelocytic leukaemia is made of disseminated fibrin clots that occur without vascular injury” [5]. Regardless of the terminology used for microthrombus formation or the setting in which thrombogenesis occurs, thrombus formation is a complex process that involves endothelial activation, integrin-mediated platelet-platelet and platelet-neutrophil aggregation, and formation of cross-linked fibrin [9]. These cellular processes are promoted by the adhesive functions of integrins, which are regulated at the levels of integrin expression, integrin activation through “inside-out” signalling, and post-ligand binding events through “outside-in” signalling [10].

The incidence of thrombosis is increased under conditions of hypoxia compared with normoxia [11, 12], and the more recent review by Chang refers to hypoxia as a characteristic of organ dysfunction in sepsis [5]. The vascular remodelling response to hypoxia is controlled primarily by hypoxia-inducible factors (HIF1 and HIF2) in nucleated cells [13]. Under hypoxia or following inflammatory challenges including the onset of sepsis, HIF1 α and HIF2 α (the hypoxia-dependent sub-units of HIF1 and HIF2, respectively) accumulate in the nucleus and form the active HIF1 or HIF2 complex, which bind to respective target genes and causes transcriptional upregulation. Endothelial HIF1 and HIF2 targets are distinct but overlapping and include factors that control coagulation, such as pro-thrombotic tissue factor (TF) and plasminogen activator inhibitor (PAI) 1 [13, 14]. Newly-formed large vein thrombus in murine inferior vena cava is severely hypoxic compared with venous blood [15] and the HIF1 α and HIF2 α isoforms are expressed in distinct spatial and temporal patterns within

the newly-formed and resolving large vein thrombus as well as in the surrounding vein wall [15–17]. Furthermore, increases in the pulmonary levels of HIF1 α and HIF2 α expression occur in association with increases in pulmonary microthrombosis in mice [18]. These observations together suggest that increases in thrombus formation could be controlled by cell-specific HIFs, but despite evidence that systemic and endothelial hypoxia stimulate thrombosis [19, 20], the roles of endothelial cell-specific HIF1 and HIF2 in thrombus formation are unknown. Future studies should aim to assess the relative contributions of HIFs in different cell types to thrombus formation using genetically-altered mouse models and models of thrombosis [21–24].

Hypoxia increases the expression and function of endothelial integrin receptors, but the role of endothelial HIFs in the regulation of integrin function and expression is also unclear. It could be hypothesised that hypoxia or endothelial cell activation following sepsis challenge lead to HIF-dependent increases in the expression or function of hypoxia-inducible integrins, thereby stimulating thrombus formation. For example, hypoxia increases endothelial adhesion to fibrinogen via integrin α V β 3 and endothelial cell-cell interactions via α V β 3 and α V β 5 [25, 26]. The expression of cytoskeletal, focal adhesion, and cytosolic proteins that regulate integrin activation may also be affected by hypoxia in a HIF-dependent manner; these integrin-activating proteins include talin [27, 28], kindlin1–3 [29, 30], and Rap1 [31, 32]. Although it has been shown that endothelial expression of adhesive integrins can be increased by hypoxia [25, 26], experimental studies in future could aim to determine whether hypoxia and endothelial inflammation lead to increases in endothelial HIF1 α and HIF2 α , which in turn increase the expression or function of hypoxia-inducible integrins, and thereby promote thrombus formation. Such studies could aim to determine whether genes encoding hypoxia-regulated integrins contain the hypoxia-responsive element required for transcriptional upregulation by HIF binding. These studies could reveal a novel signalling pathway that controls thrombus formation and could ultimately lead to investigations of the effect of targeting such a pathway

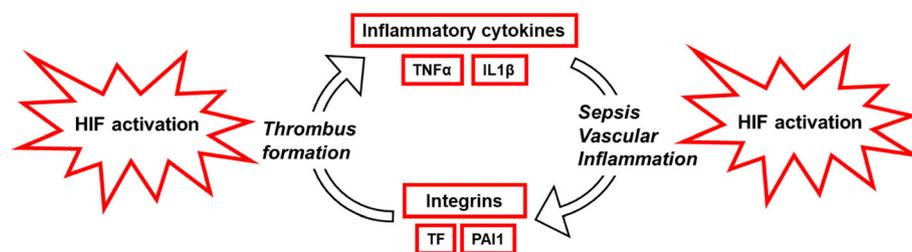


Fig. 1 HIF activation as the link between thrombus formation and inflammatory vascular injury

in humans. Although the positive associations between endothelial dysfunction, hypoxia, and thrombus formation in the septic microvasculature are well established and well described in the recent review by Chang [5], it is possible that hypoxia is both a consequence and cause of microthrombosis during sepsis and that a positive feedback loop exists between thrombus formation and inflammation in sepsis patients (Fig. 1). This possibility is consistent with the notion that pro-thrombotic pathways can also be pro-inflammatory and vice versa, but less consistent with the notion that inflammation does not alter activation of coagulation system or cause microthrombogenesis [5]. The proposal that thrombus formation could stimulate inflammation through hypoxia and HIF activation is also consistent with the observation that organ dysfunction in sepsis may be reversible [5], given that thrombus resolution is stimulated by hypoxia and activation of HIF1 and followed by temporal reductions in the severity of hypoxia and in the expression levels of HIF1 α [15, 16, 33, 34]. Future studies should aim to improve understanding of the impact of varying severities of thrombosis on sepsis-induced inflammatory injury and of the mechanisms that control sepsis-induced thrombus formation. Such studies could again use genetically-altered mouse models in combination with experimental models of sepsis and thrombosis, for example in the pulmonary microvasculature [18], which is a common site of sepsis-induced organ dysfunction.

Thrombus formation leads to hypoxia and HIF activation, resulting in the release of inflammatory factors that promote vascular inflammation, while sepsis challenge triggers HIF activation and increases the expression of integrins and coagulant factors.

Another mechanism through which hypoxia and HIF activation could promote thrombosis during sepsis is via increases in the formation of neutrophil extracellular traps (NETs) [35]. NETs are DNA fibres comprising of histones and antimicrobial proteins that are formed within the septic vasculature and promote thrombus formation [36, 37]. In his recent review, Chang postulates that “NETosis is not active hemostatic processes, but is a passive one associated with secondary event trapping blood cells and molecules such as DNAs and histones in the process of thrombogenesis” [5]. However, inflammatory and hypoxic conditions trigger HIF1 and NET activation [37, 38], and it has also been suggested that NETs themselves increase endothelial activation [39, 40], thus creating another putative thrombo-inflammatory positive feedback loop. Furthermore, it has been shown that NET formation is regulated by post-transcriptional control of HIF1 α expression following sepsis challenge and that pharmacological or genetic knockdown of HIF1 α inhibits NET deployment [35]. So, while our understanding of sepsis-induced thrombus formation has improved

substantially in recent years, future studies investigating the signalling pathways that control thrombus formation could eventually identify novel therapeutic targets and aid in the development of new therapies against thrombosis.

Abbreviations

HIF: Hypoxia-inducible factor; NETs: Neutrophil extracellular traps

Acknowledgments

None.

Author's contributions

CEE wrote the manuscript. The author read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

N/A.

Ethics approval and consent to participate

N/A.

Consent for publication

N/A.

Competing interests

The author declares that there are no competing interests.

Received: 15 July 2019 Accepted: 23 July 2019

Published online: 14 August 2019

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