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Sepsis-Induced Thrombus Formation and Cell-Specific HIFs

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Dear Editors,

The incidence of thrombosis is dramatically enhanced in sepsis patients and under conditions of hypoxia (reduced oxygenation), but the mechanisms that regulate sepsis-induced thrombosis are incompletely understood. Meanwhile, current treatments for sepsis-associated thrombosis may lead to increased bleeding or re-thrombosis. A better understanding of the mechanisms that control sepsis-induced thrombosis could lead to the development of novel treatments that aim to reduce thrombosis in sepsis patients.

Deep vein thrombosis has an annual incidence of approximately 1 in 500 in the general population [1], while the incidence of venous thromboembolism increases in sepsis patients to ~40% [2]. Mortality is also increased by more than 10% in sepsis patients with venous thromboembolism compared with thrombosis-free sepsis patients [2]. Notably, sepsis patients suffer from increased propensity for thrombosis in the pulmonary vasculature as well as the deep veins [3–6]. Sepsis not only leads to an increased risk of thrombosis in humans [1, 2], but also enhances thrombus formation in rodents [7–9]. Along with platelet-neutrophil aggregation and the formation of cross- linked fibrin, thrombosis involves endothelial activation, which is triggered by sepsis challenge [10]. Mechanisms that regulate

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Conflicts of Interest

None.

sepsis-associated thrombus formation have been elucidated previously, including inflammatory cytokine- and toll-like receptor-induced thrombosis [7, 9, 11–13], but the effect of cell-specific and hypoxia-responsive signalling pathways on sepsis-induced thrombosis remains unclear.

Hypoxia signalling and sepsis-induced thrombosis

Thrombi are more likely to form under hypoxia compared with normoxia in experimental animal studies and humans [14–16]. Under hypoxic or inflammatory conditions, hypoxia-inducible factors 1α and 2α (HIF1α and HIF2α) accumulate and translocate to the cell nucleus, where they bind with HIFβ to form HIF1 and HIF2 respectively [17]. The active HIF1 or HIF2 complex then binds to the hypoxia-responsive element of its target genes, causing transcriptional upregulation [17]. Although HIF1α expression is reduced in the leukocytes of sepsis patients compared with healthy volunteers, this may be a consequence of chronic stimulation [18], and HIF1α expression is acutely induced by sepsis challenge in human monocytes [18] and murine macrophages [19]. Subsequently, HIF1 signalling is highly involved in the vascular response to sepsis challenge [20–22]. Cell- and species-specific expression patterns of the HIFα isoforms following acute versus chronic sepsis challenge could be investigated in future studies. Given that the vascular response to inflammatory and hypoxic stimuli is regulated by HIF1 and HIF2, future studies should also aim to investigate whether sepsis leads to increases in the production of factors that control thrombosis via increased activation of cell-specific HIFs. For example, it would be interesting to determine whether sepsis challenge leads to increases in endothelial and myeloid cell-specific HIF1α and HIF2α, which in turn increase the levels of HIF1 and HIF2 targets that control sepsis-induced thrombus formation (Fig 1). Endothelial and myeloid HIF1 and HIF2 targets include factors that are highly expressed during sepsis and regulate coagulation/thrombosis, such as pro-thrombotic tissue factor (TF) [23–25] and plasminogen activator inhibitor (PAI) 1 [26–28], and anti-thrombotic TF pathway inhibitor (TFPI) [29, 30] and matrix metalloproteinases (MMPs) 2 and 9 [17, 31, 32]. Despite evidence that systemic and local hypoxia stimulates sepsis-free thrombosis [16, 23, 33, 34], and that HIF1 activation could promote sepsis-free thrombosis [35] for instance via upregulation of TF and PAI1 [23, 36, 37], direct evidence for a role of cell-specific HIFs in sepsis-induced thrombosis is lacking.

To elucidate the roles of endothelial or myeloid HIF1α or HIF2α in sepsis-induced thrombus formation, venous [38, 39] or pulmonary [40] thrombosis could be assessed in sepsis-challenged cell-specific HIF1α or HIF2α knockout mice and compared with wild type littermates. If a HIF- mediated pathway was identified as a potential therapeutic candidate for reducing sepsis- associated thrombosis, then the effect of targeting this pathway could be investigated in prevention or treatment studies of wild type mice. Such studies could ultimately identify a cell-specific HIF- mediated pathway that regulates sepsis-induced thrombus formation and would therefore represent a putative therapeutic target. If so and given that HIF agonists and antagonists are already in clinical trials, such drugs could eventually be tested for their efficacy against sepsis-associated thrombosis in humans.

Inflammation-targeting strategies in sepsis-induced thrombosis

Future studies could also aim to assess whether sepsis-associated thrombus formation can be reduced using recently-developed inflammation-targeting strategies [41]. These strategies include drug-loaded nanoparticles that are double-coated with antibodies and proteins to enable inflammation targeting and phagocytosis evasion [41]. Given that inflammatory endothelial and myeloid cells overexpress intercellular adhesion molecule (ICAM) 1 [42, 43], and that sepsis- induced thrombosis is dependent upon ICAM1 [7], therapies could be delivered not only in their free form, but also encapsulated in modified nanoparticles coated with anti-ICAM1 antibody and CD47 peptides [41]. Anti-ICAM1 antibody improves nanoparticle delivery to inflammatory ICAM1- expressing endothelial cells and macrophages, and CD47 reduces phagocytic clearance of the drug-loaded nanoparticle from the circulation [41]. Given that molecule-, cell-, and tissue-targeting strategies are currently being developed to enhance drug effectiveness against inflammatory diseases (e.g. bacterial infection [44], breast cancer [45], and autoimmune disease [46]), it would be intriguing to assess whether such nanotechnological advances could be used effectively in thrombosed tissue (Fig 2) [47].

The potential anti-thrombotic impact of signalling pathways that are identified and targeted in experimental studies of sepsis-induced thrombosis should ultimately be assessed in human cells or tissues. Nevertheless, studies employing experimental models of thrombus formation could facilitate translational studies of other diseases linked with increased thrombosis, including chronic thromboembolic pulmonary hypertension [48] and lung cancer [40]. Importantly, parallel preclinical and clinical investigations of sepsis-induced thrombus formation could lead to the development of new therapies against thrombosis in patients with sepsis.

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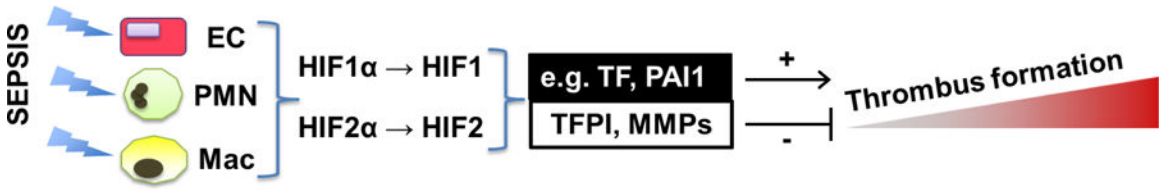

Figure 1: Investigations of HIF signalling pathways in sepsis-induced thrombosis

Proposed model of sepsis-induced thrombus formation. Abbreviations: EC, endothelial cell; Mac, macrophage; MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; PMN, polymorphonuclear cell; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

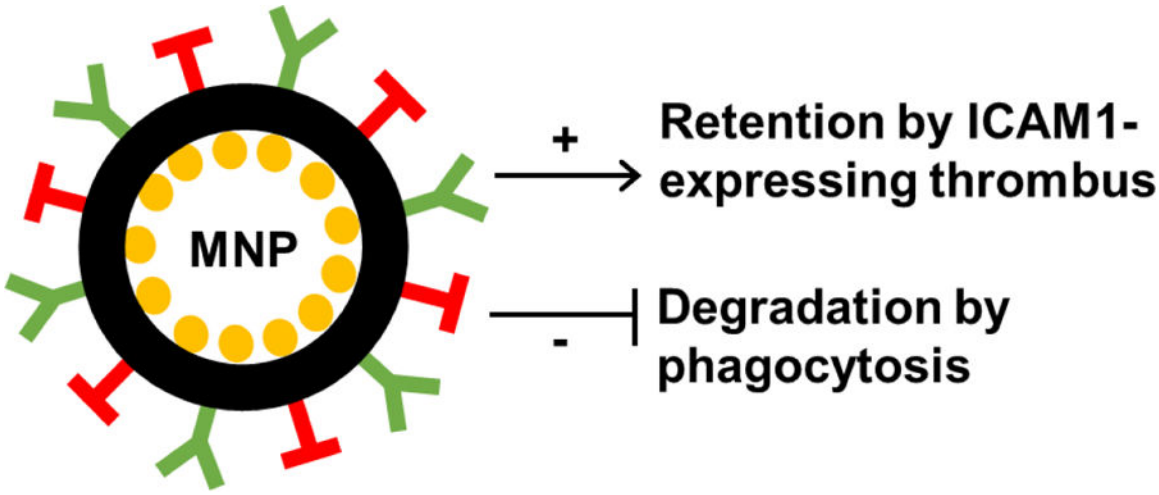

Figure 2: Potential treatment of sepsis-induced thrombosis with modified nanoparticles

Experimental studies could assess whether sepsis-induced thrombosis could be treated with drug (yellow)-loaded nanoparticles (black) modified by the additions of (i) anti-ICAM1 antibody (green) to target ICAM1-expressing inflammatory/thrombosed tissue and (ii) CD47 (red) to reduce phagocytotic clearance from the circulation. Abbreviations: ICAM, intercellular adhesion molecule; MNP, modified nanoparticle.