Endothelial cells orchestrate COVID-19 coagulopathy

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Recent data have demonstrated that coagulation activation is common in patients with severe COVID-19.[1,2] Moreover, autopsy studies have reported widespread micro-thrombi disseminated throughout the pulmonary vasculature, suggesting that vasculopathy is important in COVID-19 pathogenesis.[3] These post-mortem studies have also highlighted significant endothelial cell (EC) damage, with evidence of apoptosis and loss of tight junctions. Collectively, these data suggest that EC play a key role in orchestrating the unusual pulmonary intravascular coagulopathy (PIC) associated with SARS-CoV-2 infection.[4]

In this issue of *Lancet Haematology*, Goshua *et al* provide novel mechanistic insights into COVID-19 associated endotheliopathy (**Figure 1**).[5] In keeping with previous studies, they demonstrate that coagulation activation is common in COVID-19 patients. In addition, they further report markedly elevated plasma von Willebrand factor (VWF) levels in COVID-19 patients. This is interesting because *in vivo* biosynthesis of VWF is restricted to EC and megakaryocytes only.[6] Following biosynthesis within EC, VWF is either secreted into the plasma or else stored within intracellular organelles known as Weibel-Palade (WP) bodies. Following EC activation, this stored VWF is secreted and can tether platelets and leucocytes to the vessel wall (**Figure 1**).[6] The high plasma VWF levels associated with severe COVID-19 are thus suggestive of fulminant EC activation. Consistent with this concept, Goshua *et al* also report that plasma levels of P-selectin (which can be derived from activated platelets or following WPB secretion) are also significantly elevated in COVID-19 patients in ICU.

Several lines of evidence suggest that the high VWF levels observed by Goshua *et al* play a role in the pathogenesis underpinning COVID-19 vasculopathy (**Figure 1**). First, VWF is able to bind to platelet receptors and thus modulate platelet adhesion and aggregation.[6] Second, pathological ultra-large VWF (UL-VWF) multimers are responsible for thrombotic microangiopathy which constitutes the hallmark of thrombotic thrombocytopenic purpura (TTP). Third, UL-VWF multimers also play a role in the pathogenesis of microvasculature occlusion in children with cerebral malaria.[7] Importantly, the thrombotic microangiopathies associated with TTP and cerebral malaria share a number of similar features to that observed with severe COVID-19. Finally, VWF has recently been shown to also play a role in regulating angiogenesis.[8] Moreover, angiopoitein-2, another protein stored within WP bodies, is also important in this context.[8] This is interesting since vigorous pulmonary intussusceptive angiogenesis has been reported in patients with fatal COVID-19.[3]

Normal blood vessels are lined by an EC monolayer which plays a critical role in preventing formation of pathological thrombosis. In particular, a number of receptors expressed on EC function to promote anticoagulant pathways. Thrombomodulin (TM) is a transmembrane

receptor constitutively expressed on EC surfaces with important anticoagulant and anti-fibrinolytic activities (**Figure 1**). TM binding switches the specificity of thrombin to favor activation of anticoagulant protein C and antifibrinolytic thrombin-activatable fibrinolysis inhibitor (TAFI).[9] Goshua *at al* report for the first time that plasma levels of soluble thrombomodulin (sTM) are significantly elevated in critically ill patients with COVID-19. Although increased plasma sTM occurs in other critically ill patients, the authors demonstrate that sTM levels in this study correlate with clinical outcome. Previous studies have demonstrated that pro-inflammatory cytokines, extracellular histones and neutrophil proteases can all promote TM shedding from EC (**Figure 1**).[9] Reduced TM expression on EC surfaces would be anticipated to impair activated protein C (APC) generation, further promoting a procoagulant and pro-inflammatory local milieu within the pulmonary vasculature. Of note, the majority of ICU cases described by Goshua *et al* had been treated with tocilizumab prior to undertaking the serum biomarker assessments of endothelial function which may have blunted the magnitude of EC serum marker differences between the ICU and non-ICU group.[5] Nevertheless, these data highlight that endotheliopathy constitutes a critical factor in the pathogenesis underpinning COVID-19 vasculopathy.

Further studies will be required to define the different mechanisms through which SARS-CoV-2 infection causes such marked EC effects, particularly within the pulmonary microvasculature. Given the diffuse nature of COVID-19 pneumonia, the large volume of alveolar cavity and proximity with the pulmonary microvasculature, local inflammation and dysregulated pro-inflammatory cytokine generation are undoubtedly important in this context (**Figure 1**).[4] However other mechanisms may include complement activation, NETosis and significant hypoxia. In addition, EC cells express the ACE2 receptor that facilitates viral entry into cells and electron microscopy studies have reported SARS-CoV-2 viral particles within endothelium.[10] Irrespective of the mechanisms of EC activation and associated PIC in COVID-19, it will be interesting to see whether immunosuppressive therapy including corticosteroids and tocilizumab actually improve EC function towards reversal of *in situ* immunothrombosis and survival improvement.

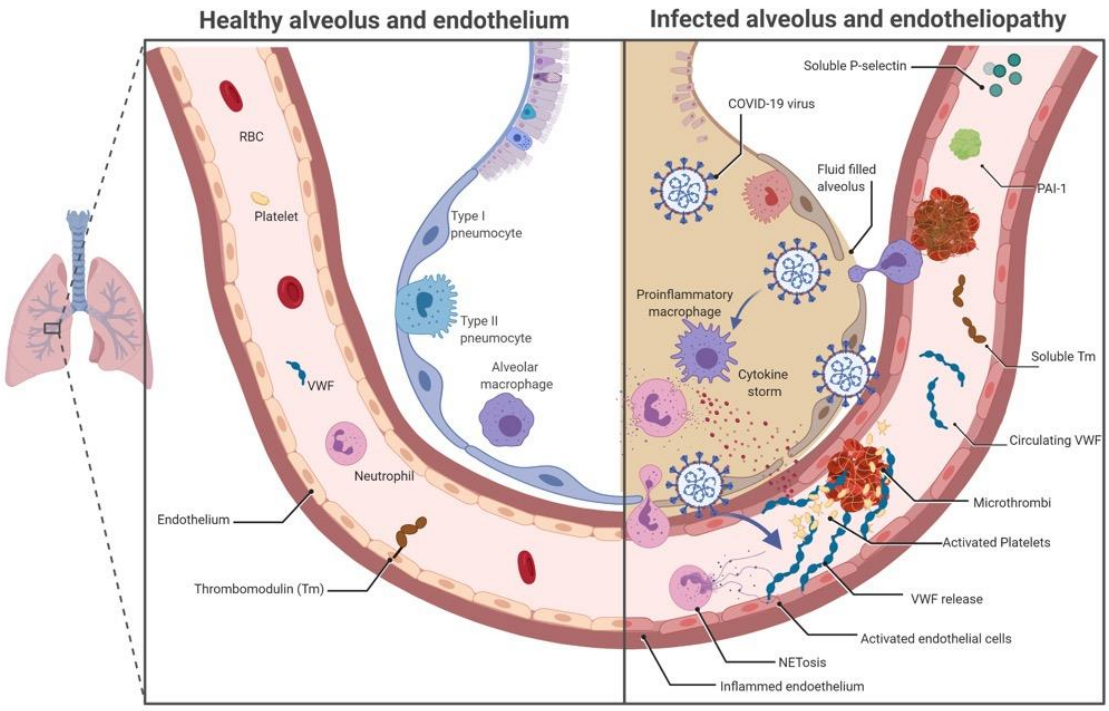

Figure 1: Endothelial cells orchestrate COVID-19 coagulopathy

Under normal conditions, the healthy alveolus and pulmonary vasculature exist in close anatomical juxtaposition. Development of thrombosis is prevented by the endothelial cell (EC) monolayer which express surface thrombomodulin (TM). Severe COVID-19 pneumonia is associated with a marked alveolar inflammatory cell infiltrate that results in systemic inflammation or a cytokine storm response. EC activation and damage are triggered though multiple pathways, including pro-inflammatory cytokines, hypoxia and complement activation. In addition, NETosis further damages local EC. As a result of this EC activation, Weibel Palade body exocytosis is triggered resulting in the release of ultra-large VWF multimers, together with P-selectin and Ang-2. The released VWF is able to bind to platelets, neutrophils and monocytes to initiate microvascular thrombosis. Concurrently, TM is shed from EC surfaces, which further promotes a procoagulant and pro-inflammatory local milieu within the pulmonary vasculature. Finally, EC damage results in separation from the basement membrane and loss of tight junctions, so that vascular permeability is markedly enhanced.

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