The Effects of SARS-CoV-2 on the Angiopoietin/Tie Axis and the Vascular Endothelium

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can cause potentially life-threatening coronavirus disease (COVID-19). COVID-19 is a multisystem disease and is associated with significant respiratory distress, systemic hyperinflammation, vasculitis, and multi-organ failure. SARS-CoV-2 causes the deterioration of numerous systems, with increasing evidence implying that COVID-19 affects the endothelium and vascular function. The endothelium is important for preserving vascular tone and homeostasis. The overactivation and dysfunction of endothelial cells are significant outcomes of severity in patients with COVID-19. The Angiopoietin 1/Tie 2 pathway plays an important role in endothelium quiescence and vessel stability. The disruption of Angiopoietin/Tie balance affects the vessel contact barrier and leads to vessel leakage, and this in turn causes endothelial dysfunction. Although vascular instability through SARS-CoV-2 is associated with endothelial dysfunction, it is still not understood if the virus affects the Angiopoietin/Tie axis directly or via other mechanisms such as cytokine storm and/or immune response associated with the infection. This review provides an overview of the impact SARS-CoV-2 has on endothelial function and more specifically on the Angiopoietin/Tie pathway.

SARS-CoV-2 endothelial cell angiopoietin Tie1/2 ACE2 inflammatory cytokines

Coronaviruses (CoVs) are a family of single-stranded RNA viruses that are a source of respiratory and digestive diseases in humans and animals [1]. The coronavirus family is separated into four different genera, i.e., *Alphacoronavirus* (α-CoV), *Betacoronavirus* (β-CoV), *Gammacoronavirus* (ϒ-CoV), and *Deltacoronavirus* (δ-CoV), which are part of the subfamily of *Orthocoronavirinae*. α-CoVs and β-CoVs cause disease in humans and other mammals, whereas ϒ-CoVs and δ-CoVs cause disease in avian species [2]. Human coronavirus (HCoV) OC43 and HCoV-HKU1 belong to β-CoV, whereas HCoV-229E and HCoV-NL-63 belong to α-CoV. These four human coronavirus species result in varying severity [3]. Severe acute respiratory syndrome coronavirus (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and, more recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belong to β-CoV genera and all of them result in more severe symptoms like pneumonia [4].

Virion Composition of Coronaviruses

The diameter of coronaviruses is about 80–120 nm in length, and the virus is surrounded by two layers of lipid molecules with envelope (E), spike (S), nucleocapsid (N), and membrane (M) proteins within the lipid bilayer. The S protein belongs to class I transmembrane proteins and is around 128–160 kDa prior to covalently attaching to carbohydrates and 150–200 kDa following N-linked glycosylation [5][6]. S protein cleaves into three identical units of

polypeptides (S1–S3) by proteases found on target cells. S1 is involved in binding to receptors and S2 for membrane fusion. The endoplasmic reticulum (ER) stress response is stimulated through the infective process of the virus by S protein, which leads to apoptosis through unfolded protein response activation [7]. The M protein has a molecular weight of 25–30 kDa and has three functional regions which span the phospholipid bilayer [8]. M protein plays a role in viral infection processes such as RNA packaging and virion formation. The E protein is a relatively small protein with a molecular weight of 8–12 kDa and is involved in the assembly and release of virions [9]. N protein has a molecular weight of 43–50 kDa and supports the packaging of RNA into ribonucleocapsids. It also plays a role in numerous activities, including regulating virus assembly by interacting with M protein and the viral genome, increasing the replication and transcription of RNA genome, and in escaping the host immune reaction. Coronaviruses have one of the largest viral RNA genomes, roughly 27–32 kilobases (kb) [10]. The 5′-end of the genome encodes non-structural proteins, including proteases and transcription factors, and the 3′-end encodes structural proteins as well as cis-acting RNA elements crucial for RNA production [11].

SARS-CoV-2 Infection

SARS-CoV-2 infection can progress into a critical and potentially fatal respiratory disorder and pulmonary damage in patients through affected upper and lower respiratory epithelial cells, including alveolar type 2 (AT2) cells. The entry of SARS-CoV-2 into the target cell is the first step in the infection process and development of disease. Entry begins with attachment of the SARS-CoV-2 S glycoprotein to the host surface angiotensin converting enzyme 2 (ACE2), followed by S protein cleavage by cell surface transmembrane serine protease 2 (TMPRSS2) into the S1 and S2 domains. The S1 subunit contains the receptor-binding domain (RBD) that recognises and binds to the ACE2 receptor, and therefore regulates virus specificity to the host cell and the infection processes that lead to disease [12]. The RBD shifts from an upright posture to connect with the ACE2 receptor to a horizontal posture to escape the immune response. After S protein binds to ACE2 on the target cell surface via its RBD, cleavage by TMPRSS2 reveals the fusion peptide on the S2 subunit to initiate the fusion process of the virus to the host cell membrane [13]. TMPRSS2 is expressed in the plasma membrane of upper airway, pulmonary, digestive, heart, prostate, and liver epithelial cells [14]. SARS-CoV-2 has also been shown to use endosomal cysteine proteases cathepsin B (Cat B) and Cat L to enter the host cell aside from TMPRSS2 [15]. Kawase M et al. (2012) implied that concurrent suppression of surface receptor TMPRSS2, Cat L, and Cat B effectively inhibits virion entry into host cells in vitro [16].

Both non-specific and virus-specific immune responses contribute towards COVID-19, with escape from the innate immune response strongly affecting viral RNA replication and progression of infection. The protein kinase B (Akt) signalling pathway, which is important for cell survival, is abnormally regulated by SARS-CoV-2 to support its survival and replication. Nuclear factor-kB (NF-kb) transcription factor has been suggested to be highly active in severe inflammation during SARS-CoV-2 infection [16]. RNA replication of the SARS-CoV-2 genome forms double-stranded RNA (dsRNA), which upregulates the cytoplasmic innate immune pathway via stimulation of melanoma differentiation-associated protein 5 (MDA5) and retinoic acid-inducible gene I (RIG-I). This in turn triggers signalling by mitochondrial antiviral-signalling protein (MAVS), leading to release of type I and type III interferons [17], resulting in the activation of antiviral interferon-stimulated genes (ISGs) in neighbouring cells via the Janus kinase/signal

transducer and activator of transcription (JAK/STAT) signalling pathway [18]. However, studies have shown that SARS-CoV-2 as well as SARS-CoV and MERS-CoV have various mechanisms to inhibit interferon-related immune responses, with the non-structural open reading frame 6 (Orf6) protein being involved in immune evasion and viral structural proteins inhibiting type I interferon-regulated antiviral immune responses [19][20].

The viral proteases 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) are also crucial for viral replication, with roles in hydrolysing the viral polyprotein. PLpro also disrupts the host non-specific immune reaction by targeting various host cell proteins [21]. RNA-dependent RNA polymerase (RdRp) is important for SARS-CoV-2 virus transcription, RNA genome replication, phenotype changes, and regulation of post-transcription of host cell gene expression [22]. SARS-CoV-2 also affects the target cell through its cytocidal effect, which promotes host cell death and lysis via an immune-regulated process. The formation of syncytia by fusion of SARS-CoV-2-infected cells, via formation of replication complexes formed through the movement of vesicles and disturbance of Golgi apparatus, has also been observed [23]. Rapid and unregulated non-specific responses to viral infection lead to a rise in inflammatory cytokines and immune cell activation in the lower respiratory tract, triggering damage to cells and tissues and thus exacerbation of COVID-19 [24].

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