Covid-19 Pathogenies: "Epithelial-endothelial-pericyte" cross-talk hypothesis

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Dear Editor,

We enjoyed the article by Cardot-Leccia et al. demonstrating apoptosis and pericyte loss (PL) in alveolar capillaries in COVID + lung [1]. This PL, with capillary and venular wall thickening in the area of DAD and in normal lung, without inflammation, and with intact epithelium and endothelium, is remarkable; we believe it supports our "Epithelial-Endothelial Cross-Talk" hypothesis [2] with additional players - pericytes.

As SARS-CoV-2 has minimal cytopathic effects and low immunogenicity [2], we believe that direct epithelial cell-injury is not the usual mechanism by which COVID-19 progresses to ARDS. Instead, we believe, "epithelial-endothelial-pericyte" cross-talk following SARS-CoV-2 infection of pulmonary alveolar epithelial cells (AEC) results in indirect activation of endothelial cells (EC) giving rise to a procoagulant-proinflammatory and profibrotic-phenotype similar to that seen in indirect-ARDS (Supplementary Fig. 1).

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Based on our model, SARS-CoV-2 infection of AEC leads to downregulation of ACE2 and increased gene-expression of IL-6 and TNF-α in AEC via SARS-CoV2-ACE2-TACE and SARS-CoV2-PRR-NF$_x$B interactions [3]. IL6-sIL-6Rα, IL-1β, and sTNF-α are then released on the luminal and abluminal sides of epithelial cells. IL6-sIL6R-1α and sTNF-α subsequently act on the EC from the basal side and increases the gene and protein expression of ACE and AT1R in the EC. Increased ACE activity on the apical side of EC increases local production of Ang II and AT1R upregulation results in increased Ang II-AT1R activity. Ang II-AT1R overactivity then increases TACE-TNF-α and ACE-AT1R expression and downregulates ACE2 in the EC, establishing a positive feed-forward pathway [3]. Increased ACE/ACE2 balance along with upregulated IL-6 and TNF-α mediated actions may stimulate a cascade of pathways, resulting in vasoconstriction, PL, endothelial barrier-disruption and cytokine-release syndrome (Fig. 1).

Direct action of Ang II-AT1R on pericytes can increase cytosolic calcium, resulting in pericyte contraction and capillary vasoconstriction. Ang II-AT1R-PKC pathway-mediated closure of intercellular gap-junctions can lead to pericyte-pericyte and pericyte-endothelial uncoupling [4]. Upregulation of Ang-2 and VEGF activity by Ang II-AT1R-MAPK pathways antagonizes protective Tie2-Ang-1 system resulting in PL, EC apoptosis and pathological angiogenesis. Increased TACE-mediated ectodomain shedding of ACE2 and Ang II-mediated reduction of ACE2 expression may relieve repression of integrin signaling rendering pericyte more susceptible to the Ang-2 and in turn, lead to the pericyte apoptosis by the Ang-2/integrin signaling pathway [5]. Ang II/AT1R/ROS regulated phosphorylation of PDGFRβ on pericytes may play a role in the pathobiology. Upregulation of ET-1 causes inappropriate pericyte contraction and reduced vessel diameter.

Our model explains the stage of PL when endothelium and epithelium are intact, perivascular inflammation is minimal and SARS-CoV2 infection of pericytes via ACE2 (even if present) seems unlikely. PL may be the first morphological change progressing to EC loss, leaving behind non-perfusing and constricted acellular capillaries. Capillary non-perfusion can stimulate intussusceptive and sprouting angiogenesis, which might explain findings wedge-shaped areas of reduced perfusion with dilatation of proximal vasculature as well as extensive angiogenesis in COVID-19.

PL and endothelial barrier-disruption can expedite pulmonary epithelium cell-injury and epithelial disruption, allowing hematogenous spread of infection. Notably, previous reports on SARS-CoV2 endothelitis have confirmed SARS-CoV2 directly infecting the EC [1].
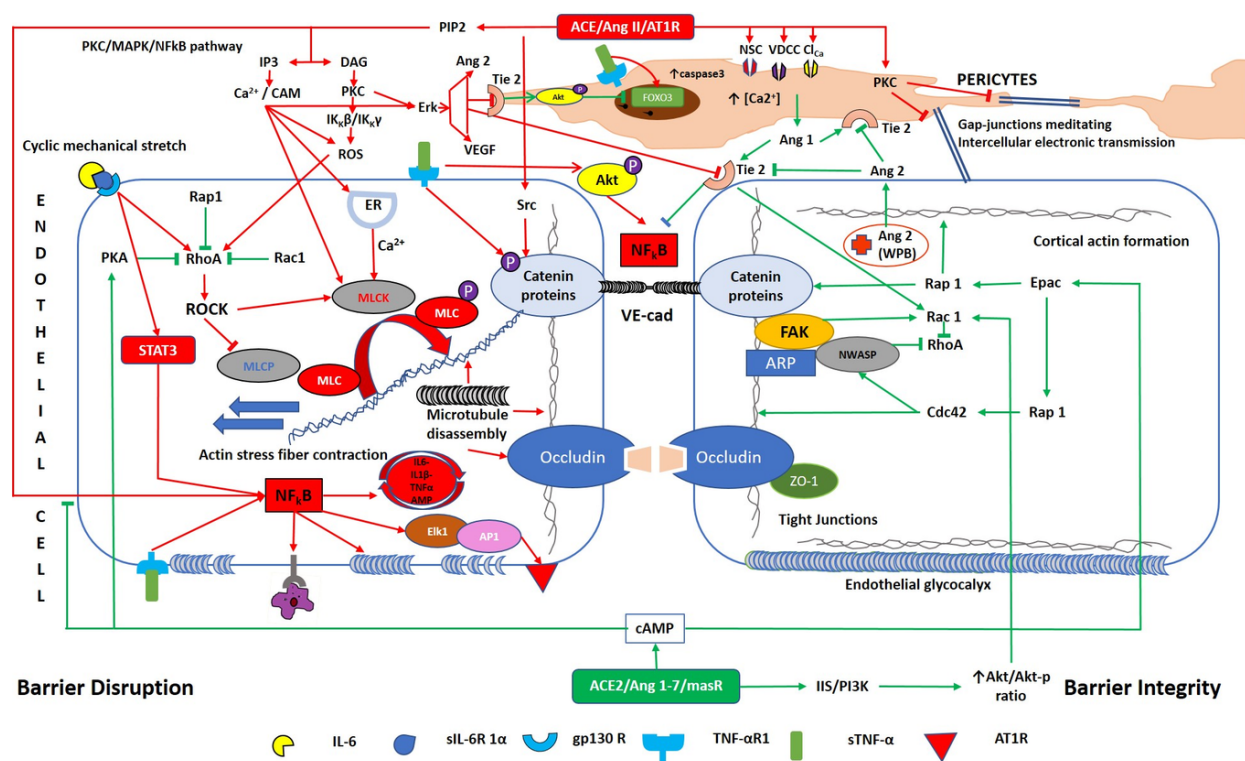
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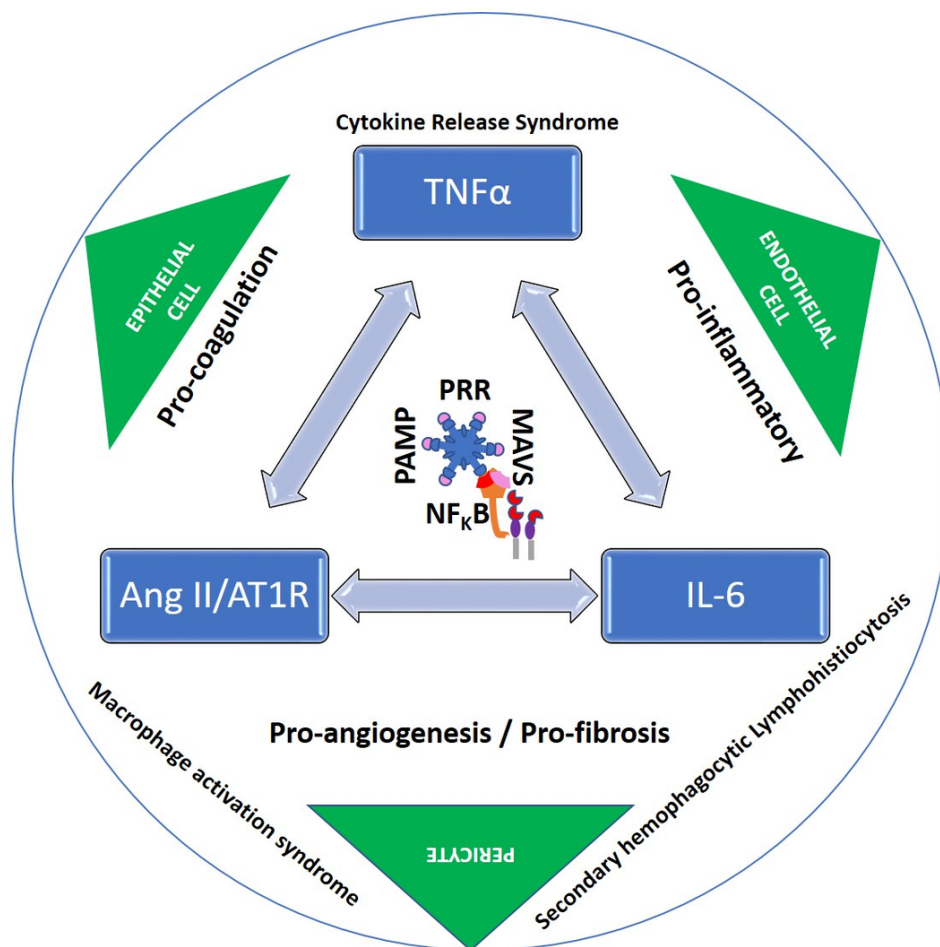
Φιγυρε1. Σιμπλε ιλλυστρατιον οφ Ανγ II-AT1P-TNΦα-IΛ6 ποσιτιε φεεδβαςκ ςψςλε ιν ΅Ο΅ΙΔ-19:

SARS-CoV-2 uses ACE2 as cell entry receptors and causes downregulation of ACE2. This is followed by host pattern recognition receptors (PRRs)-mediated detection of a viral pathogen-associated molecular patterns (PAMPs) resulting in interaction of PRRs with mitochondrial antiviral-signaling protein (MAVS) that activates NF$_\varkappa$B through a signaling cascade involving several kinases (minor direct pathway). Activated NF$_\varkappa$B translocate to the nucleus and induces the transcription of pro-inflammatory cytokines: Il-6, TNF-α and IL-1β. Binding of SARS-CoV-2 to ACE2 is also associated with activation of TACE dimer to TACE monomer. Activated TACE cleaves several membrane proteins including TNF-α, IL-6Rα, TNF-αR1 and TNF-αR2. IL6-sILRα complex produces gp130 mediated activation of STAT3 in a variety of 1L6Rα negative cells including pericytes, endothelial cells and epithelial cells resulting in full activation of NF$_\varkappa$B pathway. NF$_\varkappa$B via. downstream mediators Elk-1 and activator protein (AP-1) results in AT1R upregulation. Positive feedback pathways are stablished resulting in upregulation of ACE-AngII-AT1R, TNFα and IL6 activity mediating apoptosis and activation of pericytes, endothelial and epithelial cells and cytokine storm (major indirect pathway).

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Figure 2. Mechanisms of pulmonary endothelial barrier-disruption and pericyte apoptosis induced by ACE/Ang II/AT1R, IL6 and TNF-α-mediated pathways are illustrated (Red pathways): Endothelial barrier-disruption results from actin-myosin interaction after MLC-phosphorylation, which is regulated by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP). Activation of the actin myosin contractile apparatus disperses cortical actin and increases actin stress fibre formation, resulting in cell contraction and tensional force applied to adherens junction (AJ) proteins. RhoA acts via effector protein Rho-associated protein kinase (ROCK) to activate MLCK and inhibit MLCP. MLCK activation is modulated by Ca^{2+} which enters the cytosol from endoplasmic reticulum (ER) or extracellular space. Phosphorylation of specific tyrosine residues of cytoskeletal proteins and adhesion molecules including vascular endothelial cadherin (VE-cadherin) as well as microtubule disassembly are MLCK-independent mechanisms of barrier-disruption; Src mediated VE-cadherin phosphorylation leads to VE-cadherin internalisation. Nuclear factor ϰB (NF-ϰB) activation by Ang II, TNF-α and IL6 promotes a proinflammatory state resulting in degradation of the endothelial glycocalyx, which may expose leukocyte ligands. Ang II/AT1R activates non-specific cation channels and voltage dependent calcium channels resulting in increased cytosolic calcium in pericytes resulting in pericyte contraction and depolarization, this is coupled with opening of calcium dependent chloride channels enhancing depolarization pericyte contraction. Ang II regulates gene-expression of Ang 2 and antagonizes AngI/Tie2 pathway resulting in nuclear translocation of FOXO3A and pericyte apoptosis. TNFα increases caspase 3 activity and FOXO DNA-binding activity via p38MAPK and JNK pathways to induce pericyte apoptosis.**Downregulation of ACE2 activity results in decreased barrier enhancement activity (Green pathways):** RhoA activity is inhibited by the GTPases Rap1 and Rac1 as well as cyclic AMP (cAMP) induced protein kinase A (PKA) activation. cAMP levels increase in response Ang1-7-masR activity to induce activation of PKA (which inhibits RhoA) as well as the guanine exchange factor, exchange protein activated by cAMP (Epac). Epac (via Rap1) enhances VE-cadherin junctional integrity and actin reorganisation. Rap1 enhances barrier function via inhibition of Rho and activation of Cdc42 as well as a cooperative association with VE-cadherin. Cdc42 directly regulates cortical actin organisation and proteins including MLCK and neural-Wiskott Aldrich syndrome

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protein (N-WASP) that mediate cortical actin formation via interaction with focal adhesion kinase (FAK) and actin-related protein (ARP) thus strengthening AJ and tight junction (TJ) formation as well as cell adhesion to the extracellular matrix (ECM). FAK also signals via effector molecules to inhibit RhoA and activate Rac1. Ang1-7 acts on masR and increases the expression of Akt along with Akt phosphorylation resulting in raised Akt/phosphorylated Akt ratio leading to Rac 1 activation.