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Endothelial activation and dysfunction in metabolic syndrome, type 2 diabetes and coronavirus disease 2019

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Abstract

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 infection is a serious global concern. Increased morbidity and mortality is associated with older age, male gender, cardiovascular disease, diabetes, and smoking. As COVID-19 spreads from coastal borders, both state to state and country to country, our understanding of its pathophysiology has evolved. Age and type 2 diabetes mellitus (T2DM) play especially important roles in COVID-19 progression. T2DM is an age-related disease associated with metabolic syndrome, obesity, insulin resistance (hyperinsulinemia), hyperlipidemia, hypertension, hyperglycemia, and endothelial activation and dysfunction. This review evaluates the relationships and intersection between endothelial cell activation and dysfunction in T2DM and COVID-19. COVID-19 induces multiple injuries of the terminal bronchioles and alveolar blood-gas barrier and associated ultrastructural tissue remodeling. COVID-19 may unmask multiple vulnerabilities associated with T2DM including damage to the endothelial glycocalyx and multiple end-organ macro and microvascular diseases. Unmasking existing vulnerabilities in diabetic patients with COVID-19 is important. Globally, we must come together to better understand why T2DM is associated with increased COVID-19 morbidity and mortality.

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Introduction

Injury to the pulmonary microvascular endothelial cells (ECs) of the blood–gas barrier (BGB) may play a critical role in the pathophysiology of the novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome corona virus-2 (SARS-CoV-2 virus) infection.[1–3] EC activation and dysfunction in COVID-19 is inevitable following viral spread within the oral cavity, nasopharyngeal epithelium, bronchial epithelium and eventually the alveolar BGB lined by very thin pneumocytes (type 1 and type 2; Pn1s and Pn2, respectively) and microvascular capillaries. Type 2 diabetes mellitus (T2DM) is associated with endothelial activation and dysfunction in the affected end-organs of T2DM, resulting in neuropathy, retinopathy, nephropathy, cardiomyopathy, cognopathy and pulmonopathy (Figure 1). Perturbations of the integrity of alveolar–capillary membranes in the lung can adversely affect alveolar gas exchange. Additionally, the lung is also an end-organ of T2DM[4] and is affected by endothelial activation/dysfunction, basement membrane (BM) thickening and hyaline staining. BGB–alveolar gas exchange defects are present in diabetic patients and may be linked to rapid progression of COVID-19 requiring assisted ventilation as well as increased morbidity and mortality (Figures 1, 2).[4–7]

Pulmonary infection by SARS-CoV-2 may be perceived as an injury and healing mechanisms are induced in an attempt to (*i*) destroy the invading pathogen; (*ii*) respond to the injury as in all tissues in the body; (iii) remodel, repair, resolve and recover from this highly contagious and acute viral disease.[8–10]

Initially, SARS-CoV-2 enters the nasopharyngeal regions of the upper respiratory tract. As it infects and invades there is resulting injury to these tissues (possibly associated with loss of smell), which may progress to deeper regions of the pulmonary tissue. As the infection progresses it may travel from the bronchus to the bronchioles and the alveolar BGB, resulting in dysfunction and damage to BGB pneumocytes and ECs (Figures 3, 4). During the passage of the virus from the upper to the lower airways (viral storm) there is increasing injury. The response to injury includes innate and adaptive wound healing mechanisms. Importantly, the response to injury in the lower airways, including responses of Pn1s, Pn2s and ECs of the alveolar BGB, may not resolve because of increased viral load and replication, with newly synthesized virions compounding local innate and adaptive immune responses. Thus, completion of phases (ii) and (iii) listed in the previous paragraph may not occur. The response to continued viral replication and injury may play a significant role in the progression of hypoxygenation to pulmonary failure, which may necessitate mechanical ventilation to provide appropriate oxygen levels until the virus has been eradicated

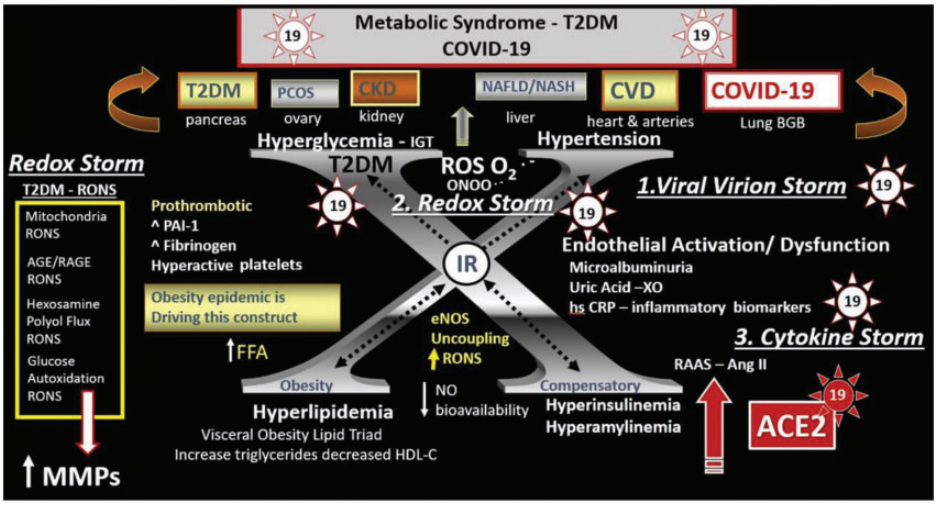

Figure 1. MetS and T2DM are associated with EC activation and dysfunction in COVID-19 patients. MetS is a constellation of metabolic abnormalities and clinical diseases associated with multiple vulnerabilities. This image illustrates the multifactorial complexities associated with MetS, impaired glucose tolerance, prediabetes, T2DM and hyperglycemia as they relate to COVID-19 and endothelial activation/dysfunction. MetS is associated with clinical diseases that are high risk comorbidities and predict poor prognosis in COVID-19 individuals both singularly and synergistically. Importantly, note the open downward arrow from the redox storm, which activates MMPs permitting increased viral replication.
ACE2, angiotensin converting enzyme 2; AGE, advanced glycation end products; Ang II, angiotensin II; CKD, chronic kidney disease; CVD, cardiovascular disease; eNOS, endothelial nitric oxide synthase; FFA, free fatty acids; hs CRP, highly sensitive C reactive protein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; MMPs, matrix metalloproteinases; NO, nitric oxide; $O_2^{\cdot-}$, superoxide; ONOO$^{\cdot-}$, peroxynitrite; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovary disease; RAAS, renin angiotensin aldosterone system; RAGE, receptor for AGE; RONS, reactive oxygen/nitrogen species; T2DM, type 2 diabetes mellitus.

and healing can occur. Additionally, there is an over-responsive redox reaction to SARS-CoV-2 infection.[11,12] Reactive oxygen/nitrogen species (RONS) lead to overwhelming damage (redox storm) and activation of the innate and adaptive immune responses (immune and cytokine storms). Together, these responses impair the response to injury resulting in progressive pulmonary failure with increased morbidity and mortality (Figures 3, 4, 5).

Wound healing in COVID-19 and histopathologic remodeling

Normal wound healing involves a dynamic and complex series of coordinated mechanisms that are outlined briefly below (Figure 5).[8–10] SARS-CoV-2 virions cause injury to cells and induce apoptosis/necrosis including loss of epithelial cells and ECs. Injury to the nasopharynx and upper and lower respiratory tracts results in bleeding, microvessel contraction, activation of complement and innate and adaptive immune responses. Infiltration of three waves of inflammatory cells occurs as follows. Mast cells/eosinophils are the first responders to injury and are important to signal the other waves of inflammatory cells; these first-wave cells are not usually noted in biopsy or autopsy fixed tissue samples but some reports have demonstrated the persistence of eosinophils.[13,14] The first wave also

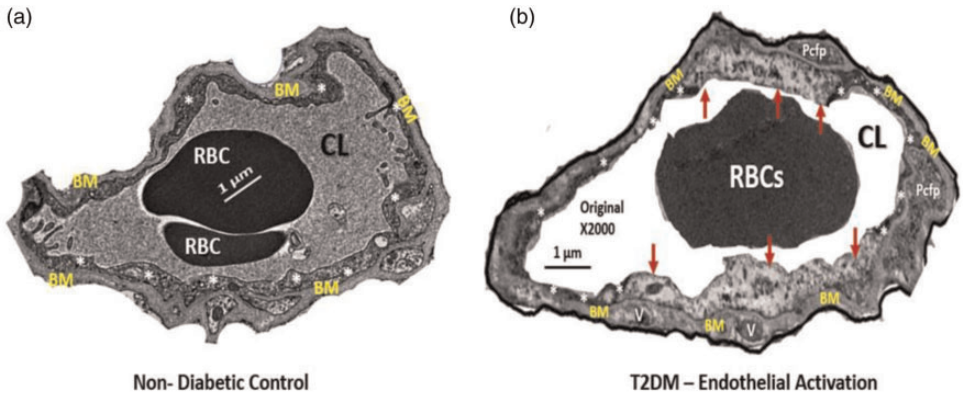

Figure 2. Example of a morphologically activated EC. Panel (a) illustrates the normal EC capillary ultrastructural morphology. Panel (b) depicts a very thinned EC cytoplasm (asterisks) with abrupt swelling and thickening of the EC cytoplasm (red arrows) indicating EC activation. Note that the BMs are thickened with vacuolization (V) observed just beneath the regions of EC thickening. Swelling with electron lucency suggested a loss of cytoplasmic organelles. These changes in ECs have also been a common finding in other end-organs of T2DM. Images (panel A and B) were captured in the cortical regions of control mice and 20 week-old obese insulin resistant T2DM db/db mice and are representative of endothelial activation. Importantly, the ultrastructural changes in T2DM as depicted in panel B are associated with EC stiffening and thoracic aortic stiffening in T2DM. Also note that the capillary in panel B has been cleaned of its plasma contents to better illustrate EC activation and changes in morphology. Original magnification $\times 2000$ with intact scale bar = 1 µm in panels A and B.
Pcfp, pericyte foot process; RBCs, red blood cells.

includes neutrophils, and is followed by the second wave of macrophages or monocyte-derived macrophages and then by a third wave of T and B lymphocytes associated with adaptive immunity. Because there is ongoing wounding occurring in close proximity, all of these cells may be observed concurrently. However, monocytes, macrophages and lymphocytes predominate for the longest period in histological examinations. Recent studies of autopsy and biopsy lung specimens have demonstrated similar histopathological findings in COVID-19 related deaths.[13,14]

Early findings in patients undergoing surgery for lung cancer who were later found to have COVID-19 at the time of surgery demonstrated early changes associated with lung infection. These included edema, proteinaceous exudates, focal reactive hyperplasia of pneumocytes in the alveolar BGB, patchy inflammatory cell infiltration, and multinucleated giant cells (usually formed by fusion and aggregation of macrophages) without prominent alveolar membranes.[13] Moreover, a patient who died despite mechanical ventilation was found at autopsy to have alveolar exudative and interstitial inflammation (primarily macrophages and monocytes) with moderate numbers of multinucleated giant cells, minimal lymphocytes (most CD4-positive), and some eosinophils and neutrophils. Focal hemorrhages with hyaline thrombi were noted in a minority of microvessels and multiple COVID-19 viral particles with classic corona spikes were observed within Pn2s.[14] While histological studies have been few, they provided insight into tissue responses to COVID-19-associated injury. Undoubtedly, we will continue to learn more as the pandemic

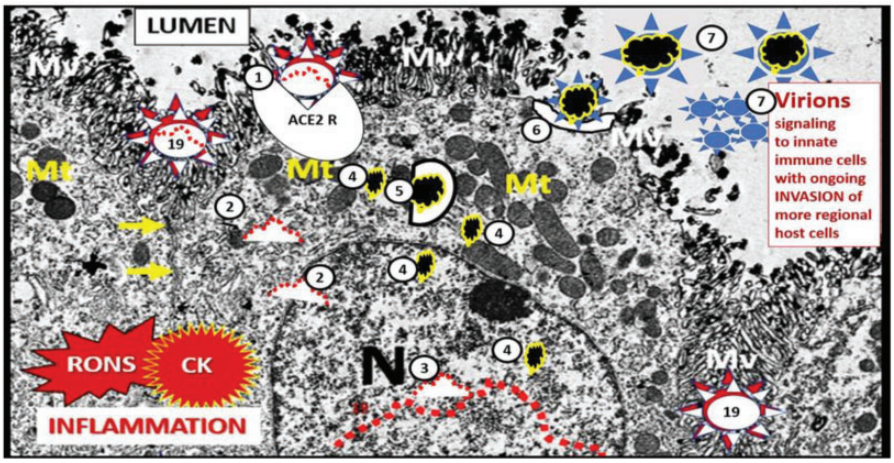

Figure 3. Potential multistep scheme (1–7) of SARS-CoV-2 infection defined using transmission electron microscopy of the terminal bronchiole. This illustration and scheme depicts SARS-CoV-2 invasion of the terminal and respiratory bronchioles, Pn2s, and ECs as well as collateral damage caused by apoptosis/necrosis, debris, redox storm, cytokine storm and new virion production from host cells. New virions infect other regional cells with loss of function via the viral storm, redox storm and cytokine storm. The image is from a 9-week old control rat. While this multistep scheme is depicted to be sequential it is certainly possible that this process may include multiple concurrent steps. (1) Invasion of cell(s) and viral load importance; (2) Release of viral RNA genome and migration to the host cell nucleus; (3) Translation and transcription of viral RNA genome; (4) Trafficking from the nucleus to the endoplasmic reticulum and Golgi apparatus; (5) Assembly of mature virions; (6) Release of mature virions via exocytosis from plasma membrane (plasmalemma); (7) Newly synthesized virions signal to innate and adaptive immune cells with ongoing invasion of additional regional host cells and viremia.
ACE2, angiotensinogen 2; Arrows, paracellular tight junctions; CK, cytokines/chemokines; Mv, microvilli N, nucleus; RONS, reactive oxygen/nitrogen species.

continues to evolve over the coming months and years.

Terminal bronchioles, respiratory bronchioles and the BGB

The bronchioles receive incoming air from the bronchi and are the final passageway terminating at the respiratory bronchiole alveolar unit (the BGB), where oxygen delivery and carbon dioxide removal occurs. The epithelium in the terminal bronchioles is cuboidal in shape with microvilli formed from columnar bronchial epithelial cells in the bronchi and numerous cilia. From the respiratory bronchioles air travels via the alveolar duct to alveolar air sacs where the thinned cytoplasm of type 1 epithelial pneumocytes permits exchange of O_2 and CO_2 at the BGB between capillary ECs whose BMs are fused with Pn1s (Figures 3, 4, 6, 7).[15–17] While Pn2s are not in direct contact with BGB endothelial cells, they play multiple important roles in the production of surfactant express the angiotensin converting enzyme (ACE)2 receptors that interact with SARS-CoV-2 (Figure 7).

Early in the COVID-19 pandemic there was controversy regarding chronic use of ACE inhibitors (ACEis) and/or angiotensin type 1 receptor blockers (ARBs) in patients with COVID-19 infections. However, this controversy has now been alleviated as a result of a recently published retrospective study from China describing the use of

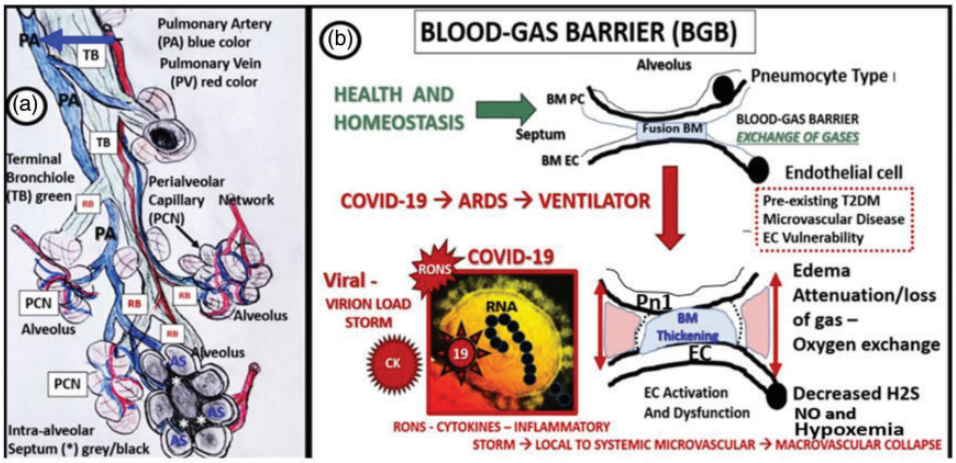

Figure 4. Terminal bronchioles and the BGB. Panel (a) is a simple illustration of the pulmonary pathway for inhaled air from the terminal bronchiole (TB) (green) to the respiratory bronchiole (RB) and the alveolar air sacs (AS) and septum (asterisks). Panel (b) depicts a simple schematic of the potential mechanisms of damage to the BGB during COVID-19 infection. It is important to note thickening of the BM of Pn1s and ECs and edema at the BGB with activation of ECs and decreased bioavailability of nitric oxide and hydrogen sulfide gasotransmitters.
ARDS, acute respiratory distress syndrome; AS, air sacs of the alveoli; BM, basement membrane; CK, cytokine; EC, endothelial cell; H_2S, hydrogen sulfide; PA, pulmonary artery (blue); PC, pneumocyte; PCN, pulmonary capillary network; Pn1, Pneumocyte type 1 cell (PC); PV, pulmonary vein (oxygenated blood in red); RNA, ribonucleic acid of SARS-CoV-2; RONS, reactive oxygen/nitrogen species; T2DM, type 2 diabetes mellitus.

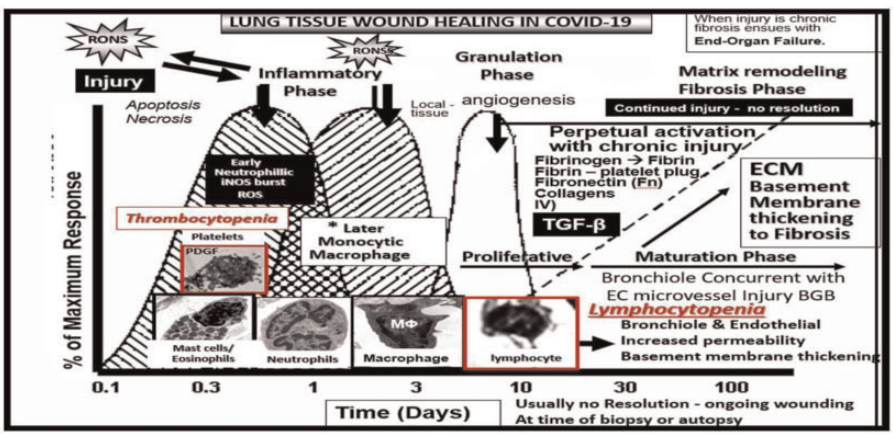

Figure 5. Potential innate wound healing mechanisms in bronchioles and the alveolar BGB. COVID-19 infections results in acute respiratory distress syndrome requiring assisted ventilation. Autopsy of the lung may show orchestrated wound healing mechanisms in response to injury, including (i) coagulation and hemostasis (not shown); (ii) inflammation; (iii) proliferation and granulation; (iv) wound repair and remodeling, and eventually, fibrosis and scarring.[8–10] Currently, autopsied tissues allow for a better appreciation of remodeling (even though samples are few in number). Recent findings regarding lymphocytopenia and thrombocytopenia (red lettering) are intriguing and may reflect an increased SARS-CoV-2 viral load. The few early images from autopsied lung tissues allow for a better understanding of this devastating disease and injury and wound healing mechanisms involved.
ECM, extracellular matrix; RONS, reactive oxygen/nitrogen species; TGF-β, transforming growth factor beta.

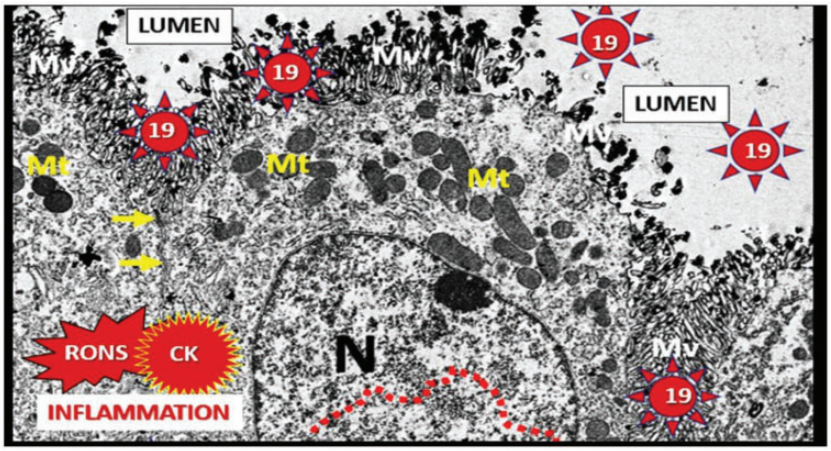

Figure 6. Terminal bronchiole from 9-week-old Sprague-Dawley rats. This image depicts a terminal bronchiole with examples of COVID-19 corona-like red spikes to illustrate how COVID-19 might invade these cells prior to or concurrently with the BGB and binding to ACE2-expressing type 2 pneumocytes. Note the presence of microvilli at the apical surface and the cell-cell tight junction yellow arrows. This image does not illustrate the decreased number of cilia in the epithelial cells of the bronchus. Arrows depict the cell–cell junctions of the tubular epithelium.
CK icon, cytokines; Mt, mitochondria; N, nucleus; Red Icon Star 19, COVID-19 virus invasion; RONS, reactive oxygen/nitrogen species.

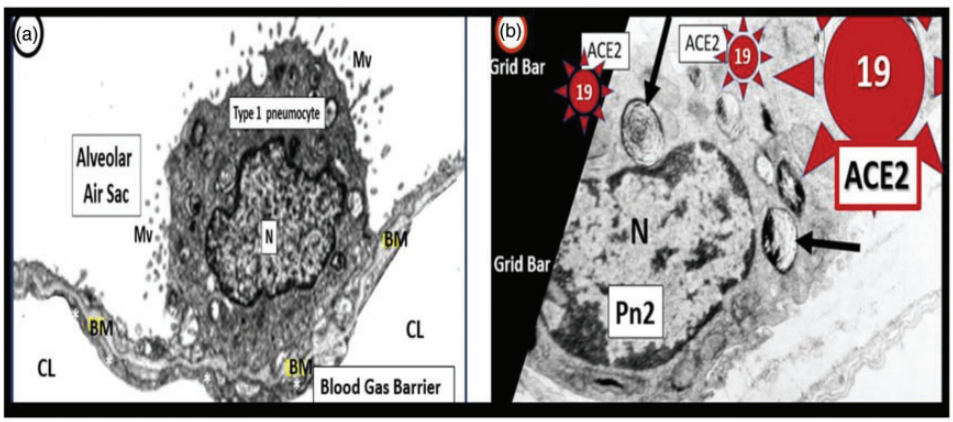

Figure 7. Type 1 and 2 pneumocytes of the alveolar BGB in 9 week-old Sprague-Dawley rats. Panel (a) depicts a single Pn1. Note how this cell abuts its BM to the BM of the inferior endothelial cell to form the BGB. Note microvilli at the apical regions of the Pn1 and the thinness of this cell's cytoplasm as it abuts the thinned cytoplasm of the inferior endothelial cell's BM. Panel (b) illustrates Pn2s and highlights morphological differences between Pn1s and Pn2s. Pn2s are characterized by morphologically distinctive phospholipid lamellar bodies (arrows) that are involved in synthesis and secretion of surfactant. Note the red star spiked icon representing COVID-19 interaction with Pn2 ACE2.
ACE2, angiotensin converting enzyme2; CL, capillary lumen of the blood-gas barrier; Mv, microvilli; N, nucleus.

ACEis and ARBs.[18] These initial concerns still need to be watched closely as the COVID-19 pandemic continues to expand, as the effects of ACEis and ARBs could vary from one ethnic group to another as well as in geographically diverse populations.

The BGB

The BGB (Figures 4, 7A, 8) comprises thin walled Pn1s and their BM, which is fused with the BM of alveolar ECs. The name "barrier" derives from the fact that blood is contained within the capillary lumen in health and this unit presents a barrier to the passage of most molecules except for diffusible gaseous elements.[19] In healthy individuals the fused BMs are extremely thin (approximately 300 nm thick) and were discovered by Frank Low in 1953 with the aid of electron microscopy.[20] The strength of this thin-walled barrier derives from the extracellular matrix composed of type IV collagen within the fused BMs.[21]

EC activation and dysfunction and vulnerable ECs in patients with T2DM

The strategically placed BGB is not only important for exchange and diffusion of O_2 and CO_2 but is also involved in the response to viral infection as a result of injury to Pn1s and Pn2s induced by local cytokines or by SARS-CoV-2 (see above). The pulmonary endothelium with its intact glycocalyx (ecGCx) may be thought of as a gatekeeper or sentinel against inflammation. Thus, endothelial activation or dysfunction, defined as a morphological change in phenotype or function in response to localized injury resulting in a dysregulated functional response, plays a central role in the inflammatory response to SARS-CoV-2 in the lung (Figures 2, 3, 4, 8).[22,23] Once activated at the BGB, the endothelium plays a central and active role in inflammatory leukocyte, erythrocyte, and platelet recruitment and adhesion via increased expression of adhesion molecules and chemoattractant cytokines and

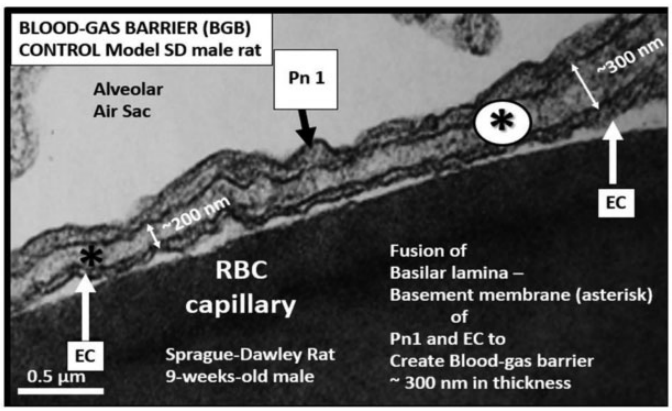

Figure 8. The BGB in male Sprague-Dawley rats at 9 weeks of age. This image depicts the BGB showing with the extremely thinned cytoplasm of Pn1s and the capillary ECs. Note that the basal lamina and BMs are fused (asterisks) between the Pn1 and ECs. This fusion of the BM allows for the rapid diffusion of oxygen and carbon dioxide; however, it also provides a barrier function that separates the blood and its constituents from the alveolar air sacs. This barrier was first defined by electron microscopy in 1953 and 1954 by Frank Low and helped to understand the roles of the BGB.

chemokines (Figure 9).[24] Thus, the pulmonary capillary endothelium of the BGB and its ecGCx plays a vital gatekeeper and antithrombotic role in maintaining the oncotic gradient across the endothelial barrier, which regulates migration and adhesion of leukocytes.

EC activation and dysfunction in COVID-19 patients

Pn2s within the alveolar BGB express ACE2 on their surface (Figures 3, 7B), the receptor for SARS-CoV-2. However, the capillary ECs that comprise the BGB express ACE2, as do arterial and venous ECs and vascular smooth muscle cells.[25] Thus, there are at least two mechanisms through which the BGB endothelium may be activated. First, the redox and cytokine/chemokine storm caused by SARS-CoV-2 invasion and damage to the terminal and respiratory epithelial cells and Pn2s may activate the endothelium. Second, direct binding of SARS-CoV-2 to ACE2 on the pulmonary endothelium of the BGB may result in EC activation and dysfunction at the BGB as well as other regional locations.[22–25]

The ecGCx of the BGB and the pulmonary epithelial glycocalyx

The intact ecGCx is essential for vascular integrity, hemostasis, signaling, blood and vessel–capillary wall interactions, and mechanotransduction.[26] The ecGCx protective coating comprises sialoproteins forming a screen-like mesh with a gel-like slime surface coating that decorates the luminal

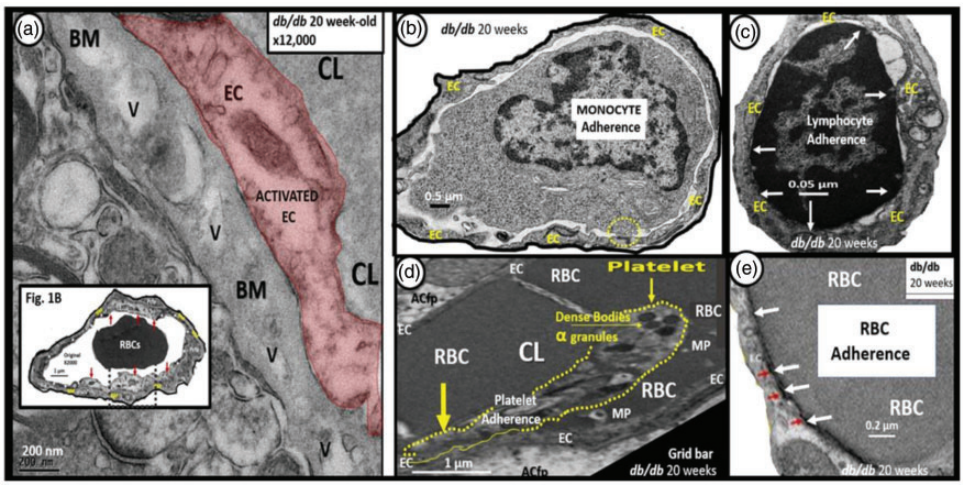

Figure 9. Examples of endothelial activation with leukocyte, platelet and erythrocyte adhesion. This multipanel image demonstrates the activated ECs and adhesion of leukocytes, erythrocytes and platelets in brain cortical tissues of a 20-week-old mouse (db/db) with obesity, insulin resistance and T2DM. Panel (a) depicts an activated EC (red) with loss of organelles and electron lucency with swelling. Note the thickened BM with vacuolization (V). Insert shows lower magnification of this same activated EC as in Figure 1B. Panels b, c, d and e are cropped images from the brain cortex of a 20-week-old db/db mouse. Panel (b) shows a monocyte adhering to the endothelium that will eventually differentiate into a monocyte-derived macrophage in the tissues. Panel (c) illustrates lymphocyte adhering to the activated endothelium. Panel (d) reveals a platelet adhering to the endothelium (outlined by a yellow dashed line). Panel (e) depicts an erythrocyte adhering to the endothelium.

Cl, capillary lumen; MP, microparticle.

side of the highly polarized endothelium. This coating is synthesized primarily by ECs, with its outermost luminal regions being supported by plasma contributions of albumin, fibrinogen and soluble plasma proteoglycans and glycolipids. The ecGCx is anchored to lipid rafts in the EC plasma membrane via three major molecules: *i*. proteoglycans (lipid rafts), *ii*. glycoproteins (plasma membrane), and *iii*. hyaluronan (CD44 anchor). These molecules also exist freely within the ecGCx without anchoring to the EC (Figures 9, 10). The ECs synthesize and secrete two protective barriers (the apical ecGCx and the basilar BM) in addition to its paracellular tight and adherens junction barriers.[27] In rodent models and healthy humans, this protective ecGCx coating prevents direct contact of blood with the plasma membranes of ECs. Furthermore, human pulmonary ECs and epithelial cells, bacteria[28] and viruses all have a glycocalyx. Coronavirus spike (S) glycoproteins are thought to have a glycocalyx consisting of glycoproteins and glycans.[29] The ecGCx, epithelial bronchioles, terminal bronchioles, respiratory bronchioles and pneumocytes are all thought to have glycocalyces. Each of these glycocalyces is thought to be in a constant state of turnover (Figures 10, 11). The ecGCx of the endothelial lining of the BGB also consists of a sugar-protein screen-like mesh coating that covers and decorates the luminal side of this highly polarized endothelium. The coating is synthesized primarily by the EC with some plasma contributions of albumin, fibrinogen and soluble plasma proteoglycans and glycolipids.

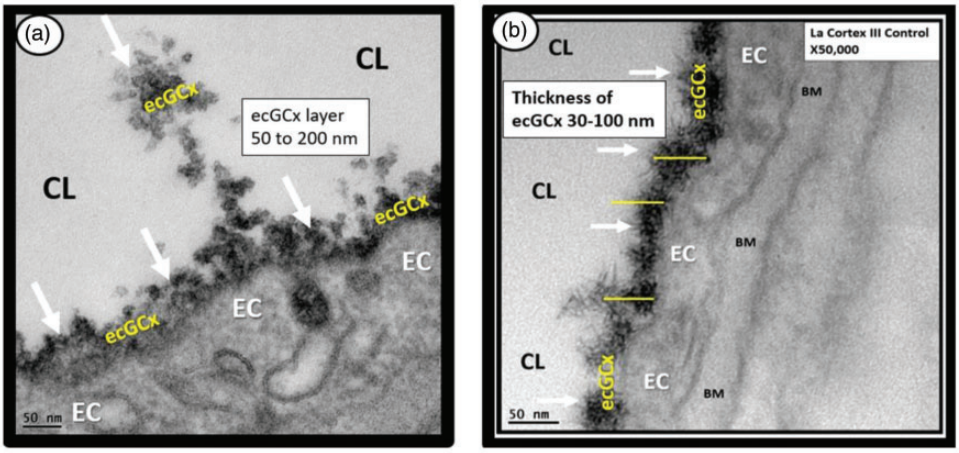

Figure 10. Example of lanthanum nitrate staining of the ecGCx in the cortical capillary neurovascular unit. Panel (a) illustrates positive (extremely electron dense) lanthanum nitrate staining of the apical surface of the EC. Note that there appears to be a base ~50-nm coating of the ecGCx with spikes up to 200 nm in thickness (arrows). Panel (b) depicts a different capillary EC in a different animal with an ecGCx base coating of 30 to 50 nm and spikes up to 100 nm. One can observe from these images that if the ecGCx were attenuated or lost that the plasma membranes of ECs would be very vulnerable to inflammation and adherence of leukocytes, erythrocytes, platelets. Similarly, the cells would be exposed to SARS-CoV-2 via trans-paracellular migration in addition to endothelial infection, resulting in activation, dysfunction and cell death. Panels (a) and (b) represent layer III capillary neurovascular unit ECs from C57/BL6 control mice that were perfusion fixed. Magnification ×40,000; scale bar of 50 nm. Panel (b): CC by 4.0.[27]
EC, endothelial cell; ecGCx, endothelial glycocalyx; CL, capillary lumen; BM, basement membrane: La, lanthanum nitrate staining.

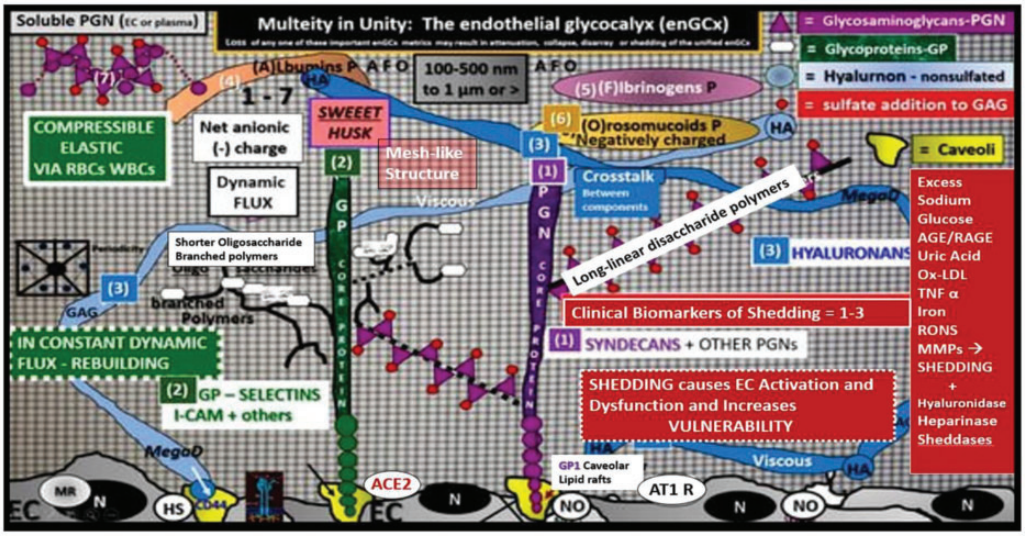

Figure 11. Illustration of the capillary ecGCx. This illustration is a simplified depiction of the highly complex electron dense ecGCx in Figure 10 and depicts the three major anchoring proteins of the ecGCx: proteoglycans (1) (purple), glycoproteins (2) (green) and hyaluronan (3) (blue). Importantly, the endothelium acts as both a structural and functional barrier. The ecGCx is in a constant state of flux, being constantly resynthesized, and is highly compressible by the blood's cellular constituents (red and white blood cells). The proteoglycans are noted for their syndecans (plus others) and the glycoproteins are noted for their selectins such as intercellular adhesion protein, P-selectin and E-selectin. Note the glycosaminoglycans, long linear disaccharides of proteoglycans, and shorter highly branched glycosaminoglycan oligosaccharides of the glycoproteins (purple triangles) that are highly sulfated (red dots). This surface layer has a net negative charge (along with additional orosomucoid proteins) and opposes movement of negatively charged molecules in the blood. This size selective semipermeable surface barrier (hindering access of proteins 70 kDa or larger) additionally serves as a glucose and sodium sink that can be rapidly overcome with attenuation and/or shedding in diabetes or clinical conditions resulting in excessive sodium chloride. The intact ecGCx contains a protective extracellular superoxide dismutase and antithrombin III while serving as a mechanotransducer. The ecGCx varies from 100 to 500 nm (300 nm mean) in thickness and from 1 to 2 μm in length. The percentage of ECs covered by the ecGCx was approximately 40% in the capillaries as shown by transmission electron microscopic studies and was greater in the brain as compared with the heart or lung lacking a blood–brain barrier.[27] In our cortical brain transmission electron micrographs using lanthanum nitrate staining we found a minimum base coating of 50 nm with thickness up to 100 to 200 nm (see Figure 10) possibly caused by multiple dehydration steps. Glucotoxicity, oxidative stress and neuroinflammation (as in the diabetic *db*/*db* model), traumatic shock injuries, ischemia/reperfusion and sepsis can result in shedding the ecGCx and/or a reduction in its volume and plasma levels of syndecans, intercellular adhesion molecules, selectins and hyaluronans. These changes can be measured clinically with elevated levels reflecting ecGCx thinning, loss or shedding.

ACE2, angiotensin converting enzyme2; AT1 R, angiotensin 1 receptor; BBB, blood–brain barrier; CD44, hyaluronan receptor of the ecGCx and cell-surface glycoprotein involved in cell–cell interactions that binds HA via the amino-terminal domain; EC, endothelial cell; GP, glycoprotein(s); H_2S, hydrogen sulfide; HA, hyaluronan; ICAM, intercellular adhesion molecule 1; MR, mineralocorticoid receptor; N, nucleus; P, protein; PGN, proteoglycans; RBC, red blood cell; WBC, white blood cell. CC by 4.0[27].

Shedding and loss of the ecGCx (sometimes referred to as the endothelial surface layer) may result from multiple metabolic toxicities associated with metabolic syndrome (MetS) and T2DM (Figures 10, 11). Such factors include excess glucose, sodium, advanced glycation end products, uric acid, oxidized low density lipoprotein cholesterol, tumor necrosis factor-α, interleukin-1β, iron, RONS, matrix metalloproteinases, heparinase and hyaluronidase.[30,31]

Recently, our laboratory has determined that staining of the capillaries of the neurovascular unit with lanthanum nitrate followed by transmission electron microscopy can reliably and reproducibly identify the ecGCx in the cortical, hippocampus and choroid plexus in rodent models of T2DM (unpublished data).[30] Other groups have shown similar findings in the brain[32] and in the lungs.[33] Importantly, Inagawa et al. demonstrated that the ecGCx of pulmonary BGB endothelial cells can be stained with lanthanum nitrate and that this endothelial surface layer is markedly attenuated and/or lost in mice treated with lipopolysaccharide. A similar mechanism may contribute to COVID-19-associated sepsis, which occurs in tandem with altered micro-hemodynamics, heterogeneous local perfusion, micro-thrombosis and endothelial dysfunction. These features may specifically contribute to increased permeability associated with interstitial fluid shift.[34] These authors also suggested that disruption of the ecGCx is causally related to the microvascular endothelial activation and dysfunction characteristic of sepsis-induced acute respiratory distress syndrome[33] in COVID-19 patients requiring intubation and assisted ventilation.

Synergy between EC activation/ dysfunction in T2DM and COVID-19 and impact on morbidity and mortality

It has recently been observed that American individuals with T2DM, obesity and MetS including dyslipidemia, insulin resistance and hypertension suffer from increased COVID-19 morbidity and mortality, especially if their condition required mechanical ventilation. This finding was observed in patients from New York City and its immediate surrounding areas, New Orleans, and Chicago. Similarly, increased morbidity and mortality were observed in a retrospective study of 201 patients with confirmed COVID-19 pneumonia from Wuhan, China[35] as well as in other locations.[36–41] These findings are extremely important because of the vast numbers of people with MetS and T2DM globally, and would undoubtedly have a great impact on the case fatality rate. T2DM is highly prevalent globally, and at the time of writing many countries have yet to experience the peak of COVID-19 incidence. Because there are no established pharmaceutical therapies for COVID-19 and care remains supportive in nature, questions surrounding potential repurposing of approved medications have arisen.

Repurposing older medications

Because there are currently no effective COVID-19 therapies, the medical profession as a whole is actively pursuing vaccine development. However, this process will take at least 1 year and during this time thousands of lives are being lost. Identification of novel pharmaceutical therapies followed by safety evaluation and clinical trials will also take a long time. The COVID-19 pandemic has driven the medical and research communities to examine approved drugs that have already undergone safety testing; their dosing, excretion rates and mechanisms of action are already established in most cases. Therefore analysis of older data may be beneficial to identify treatment modalities that prevent the rapid progression of this

very aggressive disease and its histopathologic consequences.[42]

When global society is faced with a pandemic such as COVID-19, medical doctors, scientists and researchers must employ creative approaches to identify alternative or repurposed therapies. Indeed, the repurposing or repositioning of medications to treat emerging diseases is not a new concept.[42] One example is the repurposing of sodium thiosulfate for treatment of calciphylaxis. This drug could potentially assist in treatment of COVID-19. The obesity related leptin hormone and leptin resistance may also play a role [Supplement].

Conclusion

As the COVID-19 pandemic rips through countries, major cities and small towns, it seems more and more likely that cumulative mortality will exceed that of other viral epidemics such as measles, polio, SARS-2003 (774 deaths), MERS-2012 (886 deaths), and influenza (~20,000 deaths in 2019–2020). The overall scenario may well parallel the 1918 Spanish flu pandemic (H1N1 virus). The intersection between T2DM and COVID-19 via EC activation and dysfunction, in tandem with attenuation or loss of the ecGCX, may play a significant role in morbidity and mortality.

Hill et al. sounded the alarm regarding the intersection of diabetes with COVID-19. They discussed the increased incidence of COVID-19 in patients with diabetes, the increased morbidity and mortality in diabetic patients and the importance of glycemic control in diabetic patients with COVID-19.[41]

The MetS represents a constellation and clustering of metabolic abnormalities and clinical disease states that are additionally associated with multiple vulnerabilities (Figure 1). The lung is indeed an end-organ of T2DM, a fact that becomes more evident following infection with SARS-CoV-2 as underlying vulnerabilities are unmasked. Aging and T2DM are especially important factors, as both MetS and T2DM are age-related diseases. Additionally, both older individuals and individuals with T2DM show endothelial activation and dysfunction. COVID-19 is highly associated with the aging process and multiple underlying co-morbidities including MetS, T2DM, hypertension and cardiovascular disease (Figure 1) play an important intersecting role. Multiple metabolic toxicities, including increased glucose and glycated hemoglobin A1c, are critical to assess control of T2DM. This information is crucial to the COVID-19 pandemic, its intersection with MetS and T2DM, and endothelial activation and dysfunction. The role of the protective ecGCx can now be studied with the development of a reliable and reproducible stain to identity this elusive structure under transmission electron microscopy. Understanding ultrastructural remodeling in COVID-19 should help unravel some of the mysteries associated with this pandemic. A better understanding of the ultrastructural remodeling of each cell in the respiratory tract from the oral-nasopharyngeal regions to the bronchial, terminal and respiratory bronchioles, including ACE-2-expressing Pn1s and Pn2s and ECs, will aid in understanding the COVID-19 pandemic.

Once additional pulmonary tissues from autopsy and/or biopsy specimens are available to study histopathological remodeling, researchers around the world will be able to supplement the immunological and genomic studies already completed. The question of whether individuals die with COVID-19 or from COVID-19 remains to be answered. Without careful assessment of autopsy and biopsy findings, we may not be able to answer this question. The United States population is heterogeneous[43] but particular concerns arise around the impact of COVID-19 in the African American,

Native American, and Hispanic-Latino populations because of their increased risk of MetS and T2DM.[44] Only time will reveal how these subpopulations will be affected.

During these times of great sorrow and uncertainty, we need to come together and share our research findings. Chinese clinicians and researchers have readily shared their findings. Now is the time for all societies and countries to share their findings as expeditiously as possible with regard to SARS-CoV-2 and COVID-19. We need to place our virtual hands, fingers and minds together to form 'a cup of knowledge' that will help everyone and pay homage to the unfortunate individuals and families who have lost loved ones and friends. We all must follow our countries' current recommendations to aid in the COVID-19 pandemic.

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Declaration of conflicting interest

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Supplemental material

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