SARS-CoV2 Endotheliopathy: Insights from Single Cell RNAseq

To the Editor:

We read with interest the study by Joffre and colleagues, "COVID-19-associated Lung Microvascular Endotheliopathy: A "From the Bench" Perspective" (1). The authors propose a model of endotheliopathy due to both endothelial cell severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection as well as inflammatory cytokines that likely contribute to endothelial dysfunction. In support of this model, we have previously documented that both SARS-CoV2 endothelial cell infection and the infection-associated immune activation contribute to endothelial injury and dysfunction in SARS-CoV-2–infected K18-hACE2 mice, as well as our non-human primate model and patients with severe coronavirus disease (COVID-19) (2, 3). Here, we further mined our single-cell RNAseq data from the K18-hACE2 SARS-CoV2 infected mice and independently validated viral RNA in pulmonary microvascular endothelial cells *in vivo*.

Importantly, endothelial cells with viral RNA (defined by ORF10 expression) showed increased *Ifi27l2a*, *Irf7*, and reduced *Cldn5* (Figure 1) compared with viral RNA negative endothelial cells in the same animals. Interestingly, in the K18-hACE2 model, these cells express very low levels of hACE2 compared with lung epithelial cells such as club and alveolar type II (AT2) cells (2). Increased *Ifi2712a* (or interferon, α-inducible protein 27 like 2A gene) is indicative for a type I interferon (IFN-1) response (4). Increased *Irf7* (or interferon regulation factor 7) is implicative of the upregulation of IFN-1 dependent immune responses (5). Downregulation of *Cldn5* (a critical component of endothelial tight junctions) is indicative of endothelial cell dysfunction. These data suggest that the transcriptional changes are due to the presence of viral RNA in the endothelial cells and the presence of the viral RNA is directly associated with endothelial dysfunction. We did not detect sub-genomic RNA in the endothelial cluster, which is a splice variant seen in cells that are actively replicating virus (2). Taken together these data, along with a very low level of hACE2 expression, support a model of viral transduction of endothelial cells, perhaps by exosomes or non-receptor mediated uptake of viral RNA. Further, our *in vivo* data support the *in vitro* model reported by Joffre and colleagues. ■

FeatureName	orf10+ Average	orf10+ Log2 Fold Change	*adj P Value*
orf10	6.167664858	6.336471118	4.22E–48
S	1.016107762	4.496146022	1.33E–16
N	2.523880571	4.482191851	4.44E–28
Ifi27l2a	6.244146088	2.364289062	1.39E–10
Irf7	2.398232837	1.759067722	7.81E–10
Cldn5	5.878128775	–0.733668057	0.03169382

Figure 1. TSNE plots of whole lung single cell RNAseq data in SARS-CoV-2 infected K18-hACE2 mice (Day 4 post infection). Endothelial cells were reclustered and then separated by the presence or absence of viral RNA, defined by orf10 expression. *Irf7* and *Cldn5* expression were differentially expressed in orf10 + endothelial cells. Quantitative expression data are in the table above. adj = adjusted; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TSNE = t-distributed stochastic neighbor embedding.

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Mst Shamima Khatun, Ph.D.
Tulane University School of Medicine
New Orleans, Louisiana

Xuebin Qin, Ph.D.
Tulane University School of Medicine
New Orleans, Louisiana

and

Tulane National Primate Research Center
Covington, Louisiana

Derek A. Pociask, Ph.D.
Jay K. Kolls, Ph.D.*
Tulane University School of Medicine
New Orleans, Louisiana

*Corresponding author (e-mail: jkolls1@tulane.edu).

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Reply to Khatun *et al.*

From the Authors:

We thank Khatun and colleagues for their insightful comments on our recent manuscript describing the *in vitro* effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and of coronavirus disease (COVID-19) patient sera on human endothelial cells (1). We are pleased that the authors found our proposed model of COVID-19–associated endotheliopathy to be consistent with their published data (2, 3). We apologize for not citing these important publications. In our study, we detected SARS-CoV-2 RNA in lysates of primary human lung microvascular endothelial cells (HMVEC) treated with live SARS-CoV-2 and observed that exposure to live virus induced an increase in permeability and activation of HMVEC. We also found increased permeability of HMVEC treated with sera from COVID-19 patients that correlated with disease severity. We postulated that COVID-19–associated endotheliopathy results from a combination of the actions of systemic and localized inflammatory mediators and cells on the endothelium, and by SARS-CoV-2 infection of the endothelium. Using a model of severe COVID-19 in K18-hACE2 mice, the authors previously reported SARS-CoV-2 in lung capillary endothelial cells in both the early and delayed stages of severe COVID-19, as well as lung edema, perivascular inflammation, upregulated adhesion molecule expression, and decreased vascular endothelial–cadherin expression (2, 3). Similarly, they observed co-localization of SARS-CoV-2 viral proteins with the endothelial marker CD31 in nonhuman primates with severe COVID-19, and also in autopsy samples from a patient that died of COVID-19. In their comment, the authors present additional single-cell RNAseq data on samples from SARS-CoV-2–infected mice. Their data confirm the presence of viral RNA within endothelial cells and demonstrate increased expression of *Ifi27l2a* and *Irf7*, and decreased expression of *Cldn5* by infected endothelial cells. These new data further support the concept that SARS-CoV-2 infection of endothelial cells may directly promote endothelial barrier dysfunction, and they suggest that viral infection of endothelial cells contributes to the COVID-19–associated endotheliopathy. An interesting aspect of the authors' studies is that K18-hACE2 mice have very low ACE2 expression by endothelial cells. This suggests that ACE2 may not be required for SARS-CoV-2 entry into endothelial cells and supports a model of viral transduction of endothelial cells described by the authors.

We believe that the results of our clinical-translational study and the authors' basic-translational work support our proposed multicomponent model of COVID-19–associated endotheliopathy. Furthermore, there is encouraging consistency in the effects of SARS-CoV-2 on cultured human endothelial cells (1) and *in vivo* on mouse endothelial cells (2, 3). The concordance in endothelial infection and dysfunction between the humans and mice suggests shared mechanisms of COVID-19–associated endotheliopathy. It also indicates that mouse models may lead to insights into processes that cannot be studied longitudinally in humans, such as how COVID-19 affects fixed cells in organs and tissue, including endothelial cells, tissue leukocytes and neurons, which are inaccessible in real-time in humans. Also, the apparent concordance between human and mouse endothelial cells may facilitate complementary preclinical testing of potential therapies using cultured human endothelial cells and mouse models, and even facilitate studies on effects of COVID-19 on endothelium that might predispose to the chronic effects of SARS-CoV-2 infection on the lung and other organs.

The available data suggest that the etiology of COVID-19–associated endotheliopathy involves viral infection of the endothelium, as well as the actions of systemic and localized mediators and cells on the endothelium. More work is needed to understand the mechanisms, and the relative importance of endothelial infection and of systemic and localized inflammatory cells and mediators in COVID-19. Although the specific roles of these factors in driving COVID-19–associated endotheliopathy and respiratory failure remain to be determined, collectively the data suggest that the endothelium may represent an important but understudied therapeutic target in COVID-19. ■

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Jérémie Joffre, M.D., Ph.D.
Sorbonne University
Paris, France

Michael A. Matthay, M.D.
Judith Hellman, M.D.*
University of California San Francisco
San Francisco, California

ORCID ID: 0000-0003-2278-6625 (J.H.).

*Corresponding author (e-mail: judith.hellman@ucsf.edu).

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