sensitization to overt clinical allergic disease or from one allergic condition to others (10). In this wider context, the original description of atopic dermatitis preceding allergic airway disease represents the most observed pathway. We support an expanded interpretation of the atopic march that includes the various transitions in atopic conditions as it expedites the advancement of precision practice by offering an approach to early identification of patient subgroups at risk of marching toward other allergic conditions.

There is great potential to conduct in-depth investigations of multiple phenotypes of allergic disease using the rich birth cohort data sets as presented in the current manuscript (8) with the aim of better understanding risk trajectories for the development of allergic diseases. Such information will benefit efforts toward personalized patient care and may facilitate the development of novel tailored strategies to arrest an individual's march toward further disease. Let's not "throw the atopic baby out with the bathwater" just yet. ■

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Insights into Endotheliopathy in COVID-19

Severe coronavirus disease (COVID-19) is characterized by a disruption of barrier function between the pulmonary circulation and alveoli, leading to characteristic alveolar infiltrates, hypoxemia, and in the worst case acute respiratory distress syndrome (ARDS) (1). Endothelial integrity plays an important role in maintaining the pulmonary capillary–alveolar barrier. Autopsy studies have shown that severe COVID-19 is associated with endothelial cell damage, perivascular inflammatory cell infiltration, with interstitial edema and alveolar space fluid consolidation (2). Clinical studies measuring circulating endothelial biomarkers support the hypothesis that endothelial dysfunction is an underlying factor in COVID-19 pathogenesis and a harbinger of poor outcome (3). Despite evidence that lung endothelial injury plays a key role in severe COVID-19, there has been relatively little translational investigation focusing on pulmonary vascular endothelium and its response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

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In this edition of the *Journal,* Joffre and colleagues (pp. 961–972) employed an *ex vivo* model of non–COVID-19 cadaveric primary human lung microvascular endothelial cells (HMVECs) from six non–COVID-19 donors to systematically study endothelial responses to live SARS-CoV-2 virus, inactivated virus, spike protein, and sera from patients acutely ill with COVID-19 (4). Authors demonstrated that live SARS-CoV-2 virus exposed to HMVECs directly infects and replicates within endothelial cells. This is consistent with a prior study of viral infection introduced in bioengineered human sinusoidal cells (5), and autopsy studies that have shown intracellular virus within capillary endothelium (2), Joffre's findings support a hypothesis that pulmonary capillary endothelia provide an environment for viral replication, facilitating viral access to the systemic circulation.

Authors further demonstrated that HMVECs infected with SARS-CoV-2 exhibit increased endothelial permeability as inferred using transepithelial resistance (TER). The increase in permeability was particularly sizable in three of the six HMVEC donors, although significant in all. Thus, direct pulmonary endothelial cell infection contributes to increased endothelial barrier permeability and may play a role in exacerbating pulmonary interstitial edema and transmigration of inflammatory cells. Neither co-incubation with angiotensin converting enzyme (ACE) inhibitor or inhibition of Toll-like receptors (TLRs) diminished the effect of infection-induced permeability. Nor did indirect effects of inactivated virus or spike protein alone induce permeability in HMVEC cells.

In contrast, co-incubation of virus-infected HMVECs with the ACE2 agonist diminazene aceturate led to a significant reversal of infection-induced permeability (i.e., ACE2 agonism stabilizes endothelial barrier function). This effect was observed not only in the SARS-CoV-2 model but also in an LPS-induced HMVEC hyperpermeability model. Treatment with the ACE2 agonist did not inhibit viral uptake into endothelial cells, thus precluding this as a potential mechanism. The effect of ACE2 agonism in an *ex vivo* endothelial model in the absence of circulating renin–angiotensin–system (RAS) mediators suggests that the barrier-preserving effect of ACE2 agonism on endothelial cells is linked to activation of ACE2 intracellular signaling pathways and not its effect on the RAS. This is supported by prior findings that ACE2 therapy was shown to directly antagonize vascular endothelial growth factor A (VEGFa) signaling that causes increased lung endothelial permeability (6); and VEGFa is a potent inducer of endothelial permeability (7). There is a potential protective role of ACE2 agonism in maintaining endothelial integrity in COVID-19 and sepsis, and these findings warrant further investigation.

Authors demonstrated that live SARS-CoV-2 infection of endothelial cells induce a pro-adhesive and pro-inflammatory phenotype as manifested by HMVEC surface expression of vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 2 (ICAM-2), P-selectin, platelet endothelial cell adhesion molecule 1 (PECAM-1), vascular endothelial (VE) cadherin by flow cytometry and HMVEC secretion of interleukin 6 (IL-6), VEGF, plasminogen activator inhibitor 1 (PAI-1), IL-8 and chemokine ligand 2 (CCL-2) in selected donors. Particularly notable is the association of pronounced endothelial secretion of IL-6 in HMVEC donors in whom viral infection induced large permeability. This is interesting in light of what we know about a high IL-6 phenotype of COVID-19 being associated with severe disease and ARDS (8), and the association of circulating IL-6 levels with endothelial permeability and dysfunction (9) and increased mortality (10). In addition to the well-described sources of IL-6 in COVID-19, such as inflammatory monocytes and macrophages, Joffre's finding of exuberant secretion of IL-6 from lung endothelial cells points to activated or injured lung endothelia as a substantial source of IL-6 production in severe disease. Endothelial injury with release of IL-6 may provide a feedback loop to further promote vascular endothelial activation, permeability, and pulmonary capillary leak.

Supporting the hypothesis of serum-mediated indirect activation of vascular endothelial cells, HMVECs from a single healthy donor exposed to sera from mild, moderate, and severe COVID-19 also induced increases in endothelial permeability, proportionate to disease severity. There was a similar effect on permeability when HMVECs were exposed to non–COVID-19 sera from patients with acute illness. The presence of an indirect effect of circulating COVID-19 mediators on endothelial permeability has been previously demonstrated (11). The sera-induced HMVEC permeability effect observed by Joffre and colleagues was modest when compared with the effect of direct viral infection, and similar to the effect seen with non–COVID-19 sera. One can infer that pulmonary endothelial dysfunction observed in severe COVID-19 is predominantly due to direct viral infection of pulmonary tissues and local collateral inflammatory injury, and less a function of indirect endothelial effects induced by circulating mediators.

Demonstration that human pulmonary endothelial cells *can* be infected *ex vivo* with live SARS-CoV-2 virus is, of course, not proof that pulmonary vascular endothelium is a significant reservoir for viral replication. Others have concluded that endothelial cells are resistant to infection and are unlikely significant reservoirs for productive viral replication due to extremely low surface expression of ACE2 (12, 13). Yet given that severe and fatal COVID-19 has been correlated with higher levels of viremia (14), and the route of viral migration is across the pulmonary endothelial barrier, it is certainly plausible that high viral concentration locally results in direct pulmonary endothelial infection. Antiviral therapies and SARS-CoV-2 vaccination reduce overall viral burden and thereby infer endothelial protection by decreasing viral load in perivascular spaces. And although the effect of glucocorticoids are multifactorial, they do have endothelium-stabilizing properties via direct action on endothelial glucocorticoid receptors to decrease production of IL-6 and VEGF (15), factors that Joffre and colleagues demonstrated are associated with direct infection, indirect endothelial stimulation, and increased permeability.

Major limitations of this work are those related to the study of live SARS-CoV-2 on cultured HMVEC cells from only six donors, and studying the effect of COVID-19 sera on only one HMVEC donor. As authors emphasize, endothelial responses to live infection and COVID-19 sera varied considerably across the six donors, highlighting significant host heterogeneity. Albeit donors with marked virus-induced permeability secreted correspondingly high levels of in IL-6, VEGF, and PAI-1, suggesting a hyperpermeable subphenotype that may be a target for endothelium-specific therapies. Taken together, the findings of Joffre and colleagues support the importance of pulmonary vascular endothelium in the pathogenesis of severe COVID-19, and the importance of protecting the endothelium from virus- and host-induced injury. ■

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Weaning from Veno-Venous ECMO: Lessons from 60 Years of Weaning from Mechanical Ventilation

The optimal method for weaning (or liberation) from venovenous extracorporeal membrane oxygenation (VV-ECMO) remains uncertain. In this issue of the *Journal*, Lazzari and colleagues (pp. 973–980) used a "physiological cohort" ($n = 26$) and a retrospective clinical validation cohort ($n = 638$) of patients with respiratory failure supported with VV-ECMO, to define physiological reasons for weaning failure, and to identify variables with strong weaning predictive ability (1). Patients in the physiological cohort were prospectively subjected to stepwise standardized liberation trials after fulfilling specified weaning criteria (pressure support ventilation, tidal esophageal pressure swings $\leq$15 cm H_2O, respiratory rate $\leq$30 breaths/min, pH $>$ 7.25, $Pa_{CO_2} \leq 60$ mm Hg, $Pa_{O_2} \geq 70$ mm Hg, $FiO_2 \leq 60\%$). Physiological variables and esophageal pressure swings were measured during the first liberation attempt. Weaning was considered successful if the patient maintained all physiologic values within weaning criteria at sweep gas flow of 0 L/min. Weaning was unsuccessful in 42% of trials, and 70% of weaning failures were due to excessive inspiratory effort and respiratory rate. Evaluating variables that could predict weaning outcome, baseline (before weaning sweep gas flow) $P_{ET_{CO_2}}/Pa_{CO_2}$ ratio was significantly associated with weaning success. In the univariate logistic regression, a best cutoff value of $P_{ET_{CO_2}}/Pa_{CO_2} \geq 0.84$ had a sensitivity 92%, specificity 80%, and positive likelihood ratio of 4.6. Using the clinical cohort for external validation, the ratio was again strongly associated with weaning outcome. However, only 58% of patients were correctly classified (compared with 86% in the physiologic cohort), and sensitivity and specificity were considerably lower (54% and 66%, respectively). The authors concluded that the $P_{ET_{CO_2}}/Pa_{CO_2}$ ratio was significantly associated with weaning failure in the physiological and validation cohort and could serve as a tool to assess readiness to wean in patients supported with VV-ECMO.

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