Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

tied values in Mann-Whitney-Wilcoxon test and a *P* value indicating statistical significance ($P = .0475$) was obtained. The *P* value proposed by Li and Liu[1] is derived from chi-square test. Chi-square test is more appropriate when the response (CT improvement) is a nominal variable and it is designed to test whether 2 distributions are from the same population, and hence may not be appropriate for checking whether a distribution shifts to higher values compared with another distribution. Therefore, Mann-Whitney-Wilcoxon test is more suitable here and our conclusion was valid.

In conclusion, the comments made by the readers further underline the great importance of randomized controlled trials for evaluating the efficacy and safety of novel treatments for COVID-19. Future trials involving larger population to assess with ruxolitinib or other JAK1/2 inhibitors in patients with COVID-19 are needed.

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This work was supported by the Emergency Research Project of Tongji Hospital (to J.Z.) and the Emergency Research Project of Tongji Hospital of Huazhong University of Science and Technology (grant no. 2020kfyXGYJ045 to J.Z.).

Disclosure of potential conflicts of interest: The authors declare that they have no relevant conflicts of interest.

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Available online September 18, 2020.
https://doi.org/10.1016/j.jaci.2020.07.037

Pulmonary edema in COVID-19: Explained by bradykinin?

To the Editor:

We read with great interest the article by Hosoki et al,[1] which provides an excellent overview of the mechanisms of coronavirus disease 2019 (COVID-19). However, the authors did not discuss the possible role of bradykinin in COVID-19.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to target cells through the angiotensin-converting enzyme-2 (ACE-2) receptor.[2] These receptors are expressed on epithelial cells of the lung, kidneys, intestine, and blood vessels.[3] Recently, a proposal by Veerdonk et al[4] shed new light on the role of ACE-2 in the pathophysiology of COVID-19 through the kallikrein-kinin system. ACE converts angiotensin I into angiotensin II by the removal of 2 peptides, which induces vasoconstriction and inactivates bradykinin, a known vasodilator. ACE-2 is suggested to counteract ACE in the Renin-angiotensin system (RAS) by converting angiotensin II into a metabolite, angiotensin 1-7, that leads to vasodilatation by stimulation of nitric oxide synthase.[5] Interestingly, ACE-2 also hydrolyzes the active metabolite of bradykinin: des-Arg^9-bradykinin, which binds to bradykinin receptors type 1 (BKB1), that are expressed on endothelial cells in the lungs on bronchiolar exocrine cells and pneumocytes type II. Signaling through the BKB1 receptor can induce fluid extravasation and recruitment of leucocytes to the lungs.[6] Suppression of ACE-2 by SARS-CoV-2 will impair the inhibition of des-Arg^9-bradykinin (Fig 1). Consequently, increased activation of BKB1 receptors will lead to extra fluid transversion, which results in pulmonary edema. Lessons from hereditary angioedema, show us that activation of the bradykinin type 2 receptor (BKB2) by bradykinin itself is considered to be principally responsible for the development of edema.[7] Bradykinin is generated through the plasma-contact system, when high-molecular-weight kininogen is cleaved from plasma-kallikrein. The binding of bradykinin to the BKB2 receptor on endothelial cells causes active fluid transfer through 3 known mechanisms, which all create vascular pores. The activation of the BKB2 receptor results directly in dissolution of adherens junctions, and

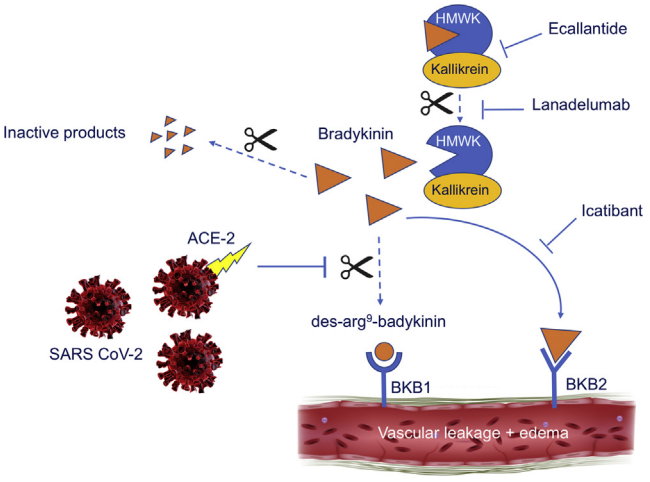

FIG 1. Proposed mechanism of increased vascular leakage and edema through activation of the BKB1 and BKB2 receptors by bradykinin, and possible therapeutic options. SARS-CoV-2 binds to the ACE-2 receptor. Bradykinin, which is generated when high-molecular-weight kininogen (HMWK) is cleaved from plasma-kallikrein, attaches to the BKB2 receptor, creating vascular leakage. Suppression of ACE-2 will impair the hydrolysis of des-Arg^9-bradykinin. Consequently, increased activation of the BKB1 receptor will lead to extra vascular leakage, resulting in pulmonary edema. Ecallantide, lanadelumab, and icatibant all target the bradykinin system and may open new therapeutic options.

also enhances phosphorylation of transmembrane vascular endothelial cadherin molecules, which are then internalized and degraded. The ensuing actin cytoskeleton constriction increases pore size between endothelial cells, with consequent vascular leakage. It is known that engagement of BKB2 by bradykinin can activate BKB1, but the overall role of BKB1 in hereditary angioedema (HAE) is uncertain. Remarkably, the BKB1 receptor is rarely expressed in normal conditions, but proinflammatory cytokines can upregulate the expression of BKB1 on endothelial cells. This suggest that blockage of BKB1 in the inflammatory state should be just as important as blocking BKB2 to prevent edema in COVID-19. To support this theory, it would be interesting to analyze whether bradykinin levels and consequently des-Arg^9-bradykinin levels are increased in patients with COVID-19. Moreover, if the pathophysiology of pulmonary edema in COVID-19 corresponds with the pathophysiology of HAE, exploring therapeutic options used to treat HAE would be a logical step. Targeting the bradykinin system by either inhibiting bradykinin production or blocking bradykinin receptors may open new therapeutic options to control COVID-19–induced pulmonary edema. Further studies are required to better understand the pathophysiology of this complex disease to invent treatment options for a more adequate response in the future.

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online September 29, 2020.
https://doi.org/10.1016/j.jaci.2020.08.038

Reply

To the Editor:

We thank Zwaveling et al[1] for appreciating our review[2] and for their insightful correspondence. They suggest that suppression of angiotensin-converting enzyme-2 (ACE-2) by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could impair the hydrolysis of des-Arg^9-bradykinin and stimulate the bradykinin receptor type 1 (BKB1) pathway to induce leakage of fluid into the lungs. In support of their hypothesis, loss of ACE-2 in an animal model aggravated acid-induced pulmonary edema, and these effects are alleviated by administration of recombinant human ACE-2.[3] In addition, a report demonstrated that SARS-CoV infection downregulates the expression of ACE-2.[4] However, other studies suggest that SARS-CoV-2 may upregulate the expression of ACE-2 in patients with coronavirus disease 2019 (COVID-19) or influenza pneumonia in alveolar epithelial cells, endothelial cells, and lymphocytes in perivascular tissue than in uninfected control autopsy lung.[5] Furthermore, single-cell RNA sequencing analysis revealed that secretory cells in the upper airway epithelium have higher ACE-2 expressions in COVID-19.[6] Thus, until the effect of SARS-CoV-2 on ACE-2 levels or functionality is thoroughly addressed in peer-reviewed publications, it is difficult to precisely determine the contributory role of ACE-2 in the bradykinin pathway during SARS-CoV-2 infection.

Zwaveling et al draw an analogy of pulmonary edema in severe COVID-19 to extravascular fluid leakage in hereditary angioedema (HAE), and hypothesized that binding of bradykinin to the bradykinin receptor type 2 (BKB2) could induce active fluid transfer through vascular pores (Fig 1, *A*). Another explanation for extravascular fluid leakage into the lungs in COVID-19 is secretion of proinflammatory cytokines such as TNF and IL-6 during the cytokine storm (Fig 1, *A*).[7] We favor a third hypothesis, where excessive and prolonged secretion of type I and type III IFNs in the airways contributes to loss of lung epithelial barrier function during COVID-19 and other RNA virus infections (Fig 1, *A*).[8,9] To test whether entry of SARS-CoV-2 through ACE-2 is sufficient to induce type III IFNs without need for viral replication, we engineered a replication-deficient SARS-CoV-2 spike-HIV-luc pseudotype virus. Infection of Caco-2 cells, which naturally express ACE-2, with this engineered virus was sufficient to increase the mRNA expression of IFN-λ2 (Fig 1, *B* and *C*), indicating that virus replication is not required for upregulating its expression. Because IFN-λ contributes to loss of lung epithelial barrier function,[8] we hypothesize that entry of SARS-CoV-2 via ACE-2 can stimulate secretion of IFN-λ and induce leakage of fluid into the lungs (Fig 1, *A*).

Zwaveling et al's hypothesis is most intriguing and certainly plausible. However, as acknowledged by the authors, there is no direct evidence showing increased levels of bradykinin or des-Arg^9-bradykinin in the patients with COVID-19 at this time. Here, we provide evidence that entry of SARS-CoV-2 through ACE-2 provides an adequate signal even without viral replication to stimulate IFN-λ2 mRNA expression, a cytokine that can cause damage to the epithelial barrier. Further investigations are required to test the hypotheses outlined in Fig 1, *A*, which may induce leakage of fluid into the lungs in COVID-19, and identify the most important pathway(s).

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