IL-33 Depletion in COVID-19 Lungs

To the Editor:

IL-33 is an alarmin that plays an integral role in lung homeostasis through its actions in wound repair, fibrosis, and remodeling processes.[1] Stored in the nucleus, IL-33 is released to the cytoplasm and extracellular fluids following insult or damage that was induced by various infectious, noxious, or environmental agents.[2] In addition to its role in allergic asthma,[3,4] studies have demonstrated elevated IL-33 in COPD plasma,[5] COPD airways,[1] and idiopathic pulmonary fibrosis (IPF) lung tissues[6]; however, a comparative analysis of lung IL-33 expression in the setting of infectious sequalae are lacking. Infection with SARS-CoV-2 causes an inflammatory cascade that results in reduced diffusion capacity, hypoxia, and death.[7,8] The Rapid Evidence Appraisal for COVID-19 Therapies (REACT) COVID investigators screened serum from 100 subjects with COVID-19 for cytokines (ie, IL-6, tumor necrosis factor, IL-8, IL-1ß, granulocyte-macrophage colony-stimulating factor, IL-33, interferon-ɣ, IL-10) and found that increased serum IL-33 levels (as well as tumor necrosis factor) were independently predictive of poor outcomes with SARS-CoV-2 in patients <70 years old (adjusted OR for IL-33, 11.14; 95% CI, 1.01-123.72).[9] The objective of this study was to characterize IL-33 expression in the lungs of patients with fulminant COVID-19, comparing this expression with that observed in other inflammatory lung diseases.

De-identified, postmortem lung sections of patients with COVID-19 (N = 8; age, 35 to 85 years; female, 25%; smokers, 50%; hypertensive, 87.5%; obese, 50%; diabetic, 62.5%; vascular disease, 50%) were obtained from the University of Nebraska Medical Center institutional review board-approved lung and/or cardiology bio-banks. De-identified samples from normal human lungs ("controls") deemed unsuitable for transplantation (N = 7; age range, 19 to 59 years; female, 14.3%; smokers, 57%), IPF (N = 4; age range, 47 to 69 years; female, 25%), COPD (N = 6; age range, 57 to 64 years; female, 50%), and post-COVID fibrosis (N = 1) with limited clinical information were obtained from explanted lungs from the lung transplant bio-bank through an honest broker. Sections were stained with human anti-IL-33 (R&D Systems, AF3625, Lot #YYZ0918051, 2.5 µg/100 µL), prosurfactant protein C (Millipore Sigma, AB3786, Lot #3466551, 1:200), and vimentin (Abcam, AB 92547, Lot #GR3258719-9, 1:200). Donkey anti-goat (AlexaFluorPlus555, A32816, Lot #VB299353) and anti-rabbit (AlexaFluor488, A21206, Lot #2156521) from Thermo Fisher at 1:100 dilutions were used. Photographs (10 per lung section per patient) of lung parenchyma were taken from the entire section under a Zeiss fluorescent microscope (Zeiss Observer.Z1 [Zeiss, White Plains, NY]) at ×20 magnification. A total of 11 COVID-19 sections (each patient with one section, except three patients had two sections each, representing different regions of the lung) were included. The integrated densities (the product of area and mean gray value) of each protein were measured as single color on black background with color threshold by Image J software (version: 2.1.0/1.53c). Statistical analysis was conducted with averaged densities of each patient with Prism 9 software (version: 9.0.0) with the use of the Mann-Whitney test vs control group; a probability value of $<.05$ was accepted as statistically significant.

Tissue IL-33 expression was increased significantly in IPF (6.57-fold; $P = .0012$) and COPD (3.91-fold; $P = .0012$) compared with control subjects, whereas patients with COVID-19 had low to negligible IL-33 expression that was significantly reduced as compared with control subjects (0.03-fold; $P = .0003$) (Fig 1A-C). Costaining with prosurfactant protein C was used to assess type II alveolar epithelial cells (AEC2); vimentin stain was used to assess mesenchymal cells (ie, fibroblasts, smooth muscle cells, and endothelial cells) and macrophages (Fig 1A-B). In control subjects, IL-33 expression was predominately nuclear and localized to endothelial cells. In comparison, patients with IPF and COPD demonstrated increased nuclear and cytoplasmic IL-33 expression in endothelial cells, macrophages, and AEC2. Minimal expression of IL-33 was demonstrated in COVID-19 lungs in some vimentin$^+$ endothelial cells. Vimentin staining was increased in COVID-19 (2.15-fold; $P = .0093$) and IPF (1.74-fold; $P = .0424$) as compared with control subjects with no difference between COPD and control subjects (Fig 1D). As compared with control subjects,

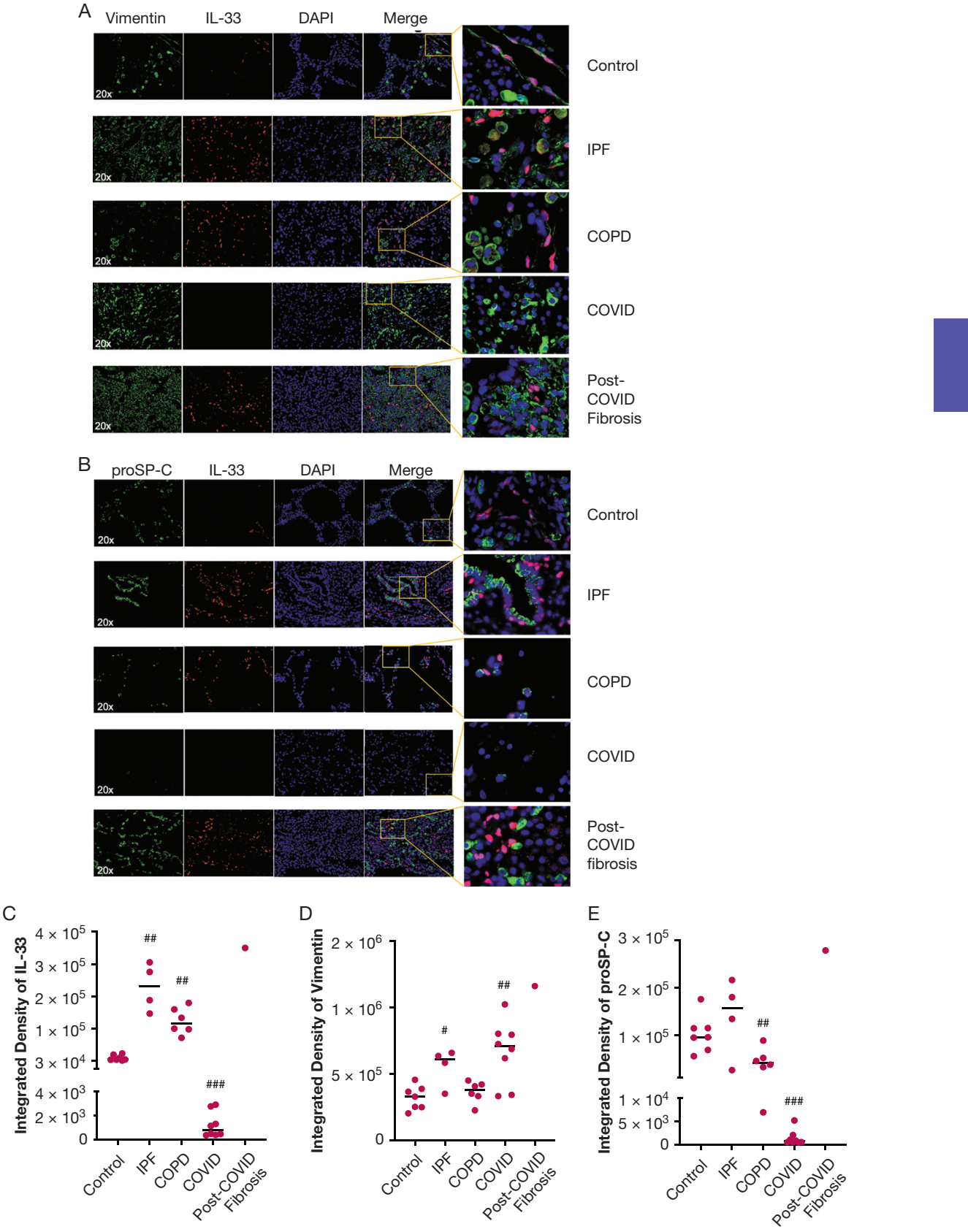

Figure 1 – *Lung expression of IL-33, vimentin and prosurfactant protein C among healthy control subjects, idiopathic pulmonary fibrosis , COPD, COVID, and post-COVID-19 fibrosis. Photomicrographs (10 per lung section per patient) were taken of the entire lung section under a (Continued)*

AEC2 numbers were decreased in COVID-19 (0.01-fold; $P = .0003$) and COPD (0.43-fold; $P = .0047$) lungs with no difference between IPF and control subjects (Fig 1E). In post-COVID fibrosis, IL-33, vimentin, and AEC2 were all increased to levels at or above that demonstrated in COPD and IPF (Fig 1A-E).

Lung tissue IL-33 was expressed in basal epithelial cells, AEC2, endothelial cells, fibroblasts, macrophages, and other progenitor cells with markedly increased expression in COPD and IPF. However, IL-33 was nearly entirely depleted from the lung tissue of subjects with COVID with negligible availability or reserve in the nucleus of any lung cell. Five of eight COVID-19 lungs demonstrated extracellular or cytoplasmic IL-33 expression, but at extremely low levels to suggest release and depletion. These findings also corresponded to a near absence of prosurfactant protein C^+ AEC2 in the COVID-19 lungs potentially to suggest cellular death and/or lack of progenitor epithelial cells to aid in lung repair and recovery processes. There was wider patient variability in vimentin expression with COVID-19, which could reflect variation in time course of COVID infection (timing unknown). Similar to COVID-19,[9] serum IL-33 levels are also increased with influenza and lung IL-33 increases in healthy control subjects who were infected with influenza.[10] However, there remains a gap of knowledge as to whether other fulminant infectious respiratory diseases are also associated with IL-33 exhaustion.

Corticosteroids are used commonly in COVID-19 and can down-regulate several cytokines,[11] but IL-33 has been recognized to be nonresponsive to glucocorticoid therapy.[3,4] Moreover, all bio-banked lungs received glucocorticoids in the standard optimization procedure before harvest. It remains possible that circulating IL-33-producing cells are also important in the response to SARS-CoV-2 infection; however, the striking depletion of lung tissue IL-33 suggests the importance of a lung compartment-specific source of IL-33. It is also noted that IL-33 is cleaved to a number of inflammatory products that potentially were not detected; however, the antibody that was used recognizes mature and cleaved forms through recognition of the Ser112-Thr270 amino acid sequence of IL-33. Nonetheless, these studies underscore the complexity of IL-33 in lung disease because lung IL-33 expression was increased strikingly in chronic lung disease, whereby serum IL-33 levels can also be elevated variably.[5,6] IL-33 is a key mediator not only in danger signaling but also in wound repair and lung recovery processes that can be marked by dysregulated fibrosis. Investigating IL-33 levels in the lung of survivors of COVID-19 would also provide insight into restoration of IL-33 in normal homeostasis. Indeed, IL-33 and AEC2 expression was increased in post-COVID fibrosis lung to support these future studies. Whether replenishment of IL-33 by lung progenitor cells as observed in chronic disease states would be beneficial or harmful in the setting of overwhelming infection is not known. In contrast, blocking viral-mediated IL-33 release early in the infectious process could be explored. When an integral role of IL-33 in Th2 diseases is considered, future studies could also use asthmatic lung samples in comparison studies. A limitation of this study is that IL-33 protein expression was assessed by immunohistochemistry because of availability; IL-33 investigations in various compartments that include lavage fluid, tissue, and serum at both protein and gene expression level to inform the role of IL-33 fully in SARS-CoV-2 are warranted.

In conclusion, these studies strengthen the relationship of IL-33 in COVID-19 to suggest that additional and longitudinal assessments are warranted to understand the mechanisms and timing of lung IL-33 expression and regulation for promoting damage or driving wound repair processes to inform potential interventional strategies.

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Figure 1 | *(Continued) Zeiss fluorescent microscope (Zeiss) at ×20 magnification. Representative images are shown with areas of interest boxed in yellow. A, Vimentin (green), IL-33 (red), and DAPI (blue) stains and a merge image with all three stains are shown for each investigated group. B, Prosurfactant protein C (green), IL-33 (red), DAPI (blue) stains and a merge image with all three stains are shown for each investigated group. Scatter plots depict median of averaged integrated densities per each patient for IL-33. C, IL-33. D, Vimentin. E, Prosurfactant protein C . The individual values are averaged from ten images per section per patient for the respective proteins. Because IL-33 was stained with both vimentin and prosurfactant protein C, 20 images (ten from vimentin and ten from prosurfactant protein C costaining) are from each subject. Statistical difference is denoted in the following manner: $^{\#}P < .05$; $^{\#\#}P < .01$; $^{\#\#\#}P < .0001$. IPF = idiopathic pulmonary fibrosis; proSP-C = prosurfactant protein C.*

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FUNDING/SUPPORT: R. Gaurav is supported by Central States Center for Agricultural Safety and Health NIOSH (U54 OH010162). Intramural UNMC COVID-19 grant (D. R. Anderson), National Institute of Environmental Health Sciences (R01ES019325 to J. A. Poole), National Institute for Occupational Safety and Health (U54OH010162 to J. A. Poole). T. R. Mikuls is supported by a VA Merit Award (CS000896) and grants from the National Institute of General Medical Sciences (U54GM115458) and the National Institute on Alcohol Abuse and Alcoholism (R25AA020818). National Institute for Occupational Safety and Health (U54OH010162 to J. A. Poole and T. A. Wyatt). T. A. Wyatt is the recipient of a Research Career Scientist Award (IK6BX003781) from the Department of Veterans Affairs. B. R. England is supported by grants from the National Institute of General Medical Sciences (U54GM115458) and the Rheumatology Research Foundation. The study was also supported by the Fred & Pamela Buffett Cancer Center Tissue Sciences Facility) Shared Resource, supported by the National Cancer Institute under award number P30CA036727.

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DOI: https://doi.org/10.1016/j.chest.2021.06.058

Acknowledgments

Author contributions: J. A. Poole takes responsibility for (is the guarantor of) the content of the manuscript, including the data and analysis. The following authors are responsible for the various aspects of the manuscript, as indicated: R. Gaurav, D. R. Anderson, S. J. Radio, B. R. England, T. R. Mikuls, G. M. Thiele, H. M. Strah, D. J. Romberger, T. A. Wyatt, J. D. Dickinson, M. J. Duryee, D. M. Katafiasz, A. J. Nelson, J. A. Poole: conception of the work; R. Gaurav, K. L. Bailey, J. A. Poole: design of the work; R. Gaurav, S. J. Radio, J. A. Poole: acquisition, analysis, & interpretation of data for the work; R. Gaurav, J. A. Poole: drafted the manuscript; D. R. Anderson, D. J. Romberger, J. D. Dickinson, M. J. Duryee, D. M. Katafiasz, A. J. Nelson: acquisition of data for the work; R. Gaurav, D. R. Anderson, S. J. Radio, K. L. Bailey, B. R. England, T. R. Mikuls, G. M. Thiele, H. M. Strah, D. J. Romberger, T. A. Wyatt, J. D. Dickinson, M. J. Duryee, D. M. Katafiasz, A. J. Nelson: performed critical analysis and revision of the draft; R. Gaurav, D. R. Anderson, S. J. Radio, K. L. Bailey, B. R. England, T. R. Mikuls, G. M. Thiele, H. M. Strah, D. J. Romberger, T. A. Wyatt, J. D. Dickinson, M. J. Duryee, D. M. Katafiasz, A. J. Nelson, J. A. Poole: approved the manuscript and are accountable for all aspects of the work.

Financial/nonfincial disclosures: None declared.

Role of sponsors: This publication's contents are the sole responsibility of the authors. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Other contributors: The authors wish to thank Lisa Chudomelka, MA, for assistance with manuscript preparation/submission.

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