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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Association of respiratory allergy, asthma, and expression of the SARS-CoV-2 receptor ACE2

To the Editor:
The novel coronavirus SARS-CoV-2 (COVID-19) was recognized in December 2019 as a cause of severe pneumonia and has now led to a global pandemic. Respiratory illnesses caused by COVID-19 cover a range of severity. The identification of risk and protective factors for disease severity from COVID-19 is critical to direct development of new treatments and infection prevention strategies. Early large case series have identified a number of risk factors for severe disease, including older age, hypertension, diabetes, cardiovascular disease, tobacco exposure, and chronic obstructive pulmonary disease. The US Centers for Disease Control and Prevention lists asthma as a risk factor for severe COVID-19 illness, which is logical given that many respiratory viruses have been well established to cause more serious illnesses in those with chronic airway diseases such as asthma. However, asthma and respiratory allergy have not been identified as significant risk factors for severe COVID-19 illness in case series from China. These preliminary reports led us to question whether we could identify features of allergy and/or asthma that could be associated with potential for reduced severity of COVID-19 illness.

SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as its cellular receptor, as do SARS-CoV and the coronavirus NL63. Higher ACE2 expression increases in vitro susceptibility to SARS-CoV, and studies examining factors that affect ACE2 gene expression have revealed that its upregulation is associated with smoking, diabetes, and hypertension, all of which are associated with increased severity of COVID-19 illness.

We hypothesized that 1 potential explanation for the unexpected observation that asthma and other allergic diseases may not be a risk factor for severe COVID-19 disease is a reduced ACE2 gene expression in airway cells and thus decreased susceptibility to infection. To test this hypothesis, we examined whether asthma and respiratory allergy are associated with reduced ACE2 expression in airway cells from 3 different cohorts of children and adults. In all 3 studies, total RNA was extracted from nasal or lower airway epithelial brush samples, with RNA sequencing performed independently for each study as previously described and provided in detail in the Supplementary Information (available in
Differential expression of ACE2 was assessed by using a weighted linear mixed effects model (limma) appropriate for RNA sequencing data and an empiric Bayes method.

Children at high risk for asthma based on parental histories and living in urban neighborhoods were enrolled prenatally and followed prospectively in the Urban Environment and Childhood Asthma (URECA) cohort; 318 of them had nasal epithelial brushes obtained at 11 years of age. Prevalence of asthma was assessed at 10 years of age, and atopic status was defined by allergic sensitization trajectories (no or minimal, low, medium, and high) as previously described. Additional type 2 biomarkers, including fractional exhaled nitric oxide, peripheral blood eosinophil level, and total IgE level, were evaluated by using standard methods. In URECA, allergic sensitization was inversely related to ACE2 expression in the nasal epithelium regardless of asthma status (Fig 1, A). In children with asthma, moderate allergic sensitization (fold change [FC] = 0.70; \( P = 4.2\text{E}^{-3} \)) and high allergic sensitization (FC = 0.54; \( P = 6.4\text{E}^{-5} \)) were associated with progressively greater reductions in ACE2 expression compared with in children with asthma but no/minimal allergic sensitization (Fig 1, B). ACE2 expression was also significantly inversely associated with type 2 biomarkers (see Table E1 in this article’s Online Repository at www.jacionline.org), including the number of positive allergen-specific IgE test results (\( \beta \) coefficient -0.089; \( P = 3.1\text{E}^{-5} \)), total IgE level (\( \beta \) coefficient -0.31; \( P = 5.1\text{E}^{-6} \)), fractional exhaled nitric oxide (\( \beta \) coefficient -0.45; \( P = 3.4\text{E}^{-3} \)), and nasal epithelial expression of IL13 (\( \beta \) coefficient -0.13; \( P = 0.07 \)). Although male sex has been associated with increased severity of COVID-19 illness, no sex-based differences in ACE2 expression were found in URECA. Of note, 10 participants reported nasal corticosteroid use at the time of nasal sampling, and it was not associated with alterations in ACE2 expression.

We also evaluated 24 adult participants with allergic rhinitis to cat who had no asthma symptoms in the prior year, were enrolled in a study in which they underwent nasal cat allergen challenge (NAC), and had been exposed to cat allergen through an environmental exposure chamber (EEC), as previously described. Pre–allergen challenge and post–allergen challenge nasal brush samples were obtained. Allergen exposure by both NAC and EEC led to significant reductions in ACE2 expression (Fig 2, A) (with NAC, FC = 0.81 and \( P = 2.4\text{E}^{-3} \); with exposure through an EEC, FC = 0.79 and \( P = 1.6\text{E}^{-3} \)).

An additional cohort of 23 adult participants with mild asthma that was not treated with asthma controller therapy underwent segmental allergen bronchoprovocation to dust mite, ragweed, or cat, as previously described. Pre–allergen challenge and post–allergen challenge bronchial brushings were obtained and
demonstrated significantly reduced ACE2 expression in lower airway epithelium in the post-allergen challenge samples (Fig 2, B) (FC 0.64; P = .01).

From in vitro models obtained from the Gene Expression Omnibus, we assessed the effects of IL-13, a type 2 cytokine strongly related to allergic asthma, on ACE2 expression in differentiated airway epithelial cells. IL-13 significantly reduced ACE2 expression (see Fig E1 in this article’s Online Repository at www.jacionline.org) in both nasal (FC = 0.44; P = 5.8E–4) and bronchial (FC = 0.80; P = 5.1E–3) epithelium.

Viral respiratory infections are the most common trigger of severe asthma exacerbations in children and adults. Unexpectedly, large epidemiologic studies of the COVID-19 pandemic in China did not identify asthma as a risk factor of severe COVID-19–related illnesses. Here, we report that respiratory allergy and controlled allergen exposures are each associated with significant reductions in ACE2 expression. ACE2 expression was lowest in those with both high levels of allergic sensitization and asthma. Importantly, nonatopic asthma was not associated with reduced ACE2 expression. Given that ACE2 serves as the receptor for SARS-CoV-2, our findings suggest a potential mechanism of reduced COVID-19 severity in patients with respiratory allergies. However, it is likely that additional factors beyond ACE2 expression modulate the response to COVID-19 in individuals with allergy, and elucidation of these factors may also provide important insights into COVID-19 disease pathogenesis.

The strengths of our study include carefully phenotyped cohorts of children and adults. Further, the allergen challenge studies included both upper and lower airway samples, with each demonstrating a consistent impact on ACE2 expression. The limitations include lack of clinical information to directly link ACE2 expression to SARS-CoV-2 infection and illness severity in our study populations. In addition, we do not have data on the ACE2 protein levels to confirm the gene expression data, although previous work suggests a direct association between ACE2 mRNA levels and ACE2 protein levels in the lung.

It is important to note that early data in the United States suggest a higher rate of asthma in patients hospitalized for severe COVID-19 illness, but the data do not specify whether the asthma was allergic, which is an important differentiation that relates to our findings. Nor do the data mention the potential presence of other comorbidities, such as obesity, that have been identified as risk factors for COVID-19 illness. Future studies focused on respiratory allergy, asthma, and perhaps other allergic disorders are needed to provide greater understanding of the impact of underlying allergy on COVID-19 susceptibility and disease severity. The modulation of ACE2 expression by type 2 inflammatory processes suggests the need to comprehensively evaluate the role of type 2 immune regulation in COVID-19 pathogenesis. Further elucidation of these relationships could identify novel therapeutic strategies to more effectively control this pandemic.

Daniel J. Jackson, MD William W. Busse, MD Leonard B. Bacharier, MD Meyer Kattan, MD J. O’Connor, MD Robert A. Wood, MD Cynthia M. Visness, PhD Stephen R. Durham, MD David Larson, PhD

REFERENCES

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention [e-pub ahead of print]. JAMA https://doi.org/10.1001/jama.2020.2648. Accessed March 25, 2020.
3. Ica HP, Look DC, Shi L, Hickey M, Powe L, Netland J, et al. Nasal allergen challenge and environmental exposure chamber challenge: a randomized trial comparing clinical and biological responses to cat allergen. J Allergy Clin Immunol https://doi.org/10.1016/j.jaci.2020.02.024. Accessed March 25, 2020.
4. Brake SJ, Barnsley K, Lu W, McAlinden KD, Eapen MS, Sohal SS. Smoking upregulates angiotensin-converting enzyme-2 receptor: a potential adhesion site for novel coronavirus SARS-CoV-2 (COVID-19). J Clin Med 2020;9:841.
5. Larson DR, Patel P, Salapatek AM, Conron P, Wilson DL, Fina A, et al. Nasal allergen challenge and environmental exposure chamber challenge: a randomized trial comparing clinical and biological responses to cat allergen. J Allergy Clin Immunol https://doi.org/10.1016/j.jaci.2020.02.024. Accessed March 25, 2020.
6. Bacharier LB, Beigelman A, Calatroni A, Jackson DJ, Gergen PJ, O’Connor GT, et al. Longitudinal phenotypes of respiratory health in a high-risk urban birth cohort. Am J Respir Crit Care Med 2019;199:71-82.
Type I IFN immunoprofiling in COVID-19 patients

To the Editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes coronavirus disease 2019 (COVID-19), is characterized by a wide spectrum of disease encompassing asymptomatic carriage, mild to severe upper respiratory tract illness that can evolve into respiratory failure, or rapidly progressing severe viral pneumonia with acute respiratory distress syndrome. Disease severity depends on viral strain, and host risk factors have been identified such as age and male sex. In addition, an excessive immune response has been identified in patients showing a cytokine storm associated with acute respiratory distress syndrome. Various immunosuppressive drugs, including IL-6 blockers or Janus kinases (JAK)-signal transducer and activator of transcription signaling inhibitors, have been suggested for the treatment of SARS-CoV-2 infection, whereas additional clinical trials are evaluating the use of recombinant IFN to foster host antiviral response (clinical trials NCT04315948 and NCT04293887). Type I IFNs (IFN-I) are major components of the innate immune system and represent critical antiviral molecules. To date, IFN-I response has not been evaluated in patients with COVID-19 and its contribution to the viral control and inflammation is unknown.

In this study, we assessed the kinetics of plasma IFN-I in patients with COVID-19 with a spectrum of severity degree. This study was approved by an ethical committee for biomedical research (Comité de Protection des Personnes HCL) (see text and this article’s Methods section in the Online Repository at www.jacionline.org).

First, we explored 3 patients issued from the first COVID cluster diagnosed in France (Les Contamines, Haute Savoie, France) in February 2020. We took advantage of the new digital ELISA technology single-molecule arrays (Simoa) and analyzed the kinetics of plasma inflammatory cytokines. IL-6, C-reactive protein (CRP), and IFN-γ-induced protein 10 (IP-10) were elevated in the 2 symptomatic patients (patients 1 and 3) (see Fig E1 in this article’s Online Repository at www.jacionline.org). Strikingly, no IFN-α2 was detectable in these 2 patients. In contrast, IL-6, CRP, and IP-10 remained low during the hospital isolation stay for the asymptomatic individual and a significant elevation in plasmatic IFN-α2 was observed. Viral loads were low, with no obvious quantitative difference between all 3 patients.

We further explored a larger cohort of 26 critically ill patients with COVID from 1 of the intensive care unit at Hospices Civils de Lyon (Lyon, France). Of note, all the patients were treated with standard of care and none received antiviral or immunotherapies. Considering the first 28 days of infection, more than half of critically ill patients required invasive mechanical ventilation (14 of 26). We observed that patients demonstrated a peak in IFN-α2 at day 8 to 10 of symptom onset corresponding to the viral replication phase, which decreased overtime to low but still detectable IFN-α2 concentrations. Conversely, a subset of patients (n = 5 [19%]) presented with sustained abrogation of IFN-I production (Fig 1, A). Simoa IFN-α2 measurement demonstrated a positive correlation with IFN-stimulated genes (see Fig E2, A, in this article’s Online Repository at www.jacionline.org) as already shown in viral infections. We noticed a strong proinflammatory response in all cases (CRP, IL-6, or IP-10), which started early and remained positive, whereas IFN-I response decreased after day 10 of infection (Fig 1, B-D). Patients with no IFN-α production presented poorer outcome, all of them requiring invasive ventilation (n = 5 of 5) and showing a longer intensive care unit stay (Table 1). The viral load tended to be higher in IFN-negative patients with COVID-19 at disease diagnosis. IFN-β and IFN-λ were undetectable, whereas low amount of IFN-γ was detected in all patients with no evident link with IFN-α2 level (see Fig E2, B-D).

Taken together, our data demonstrate a heterogeneous pattern of IFN-α response in patients with COVID-19, with IFN-I response being impaired in about 1 of 5 of critically ill patients. This defective innate immune response may be associated with a poor outcome. In murine models of SARS-CoV-1 infection, delayed IFN-I production is associated with lung lesions and fatal outcome whereas early administration of IFN-I prevents lung lesions. SARS-CoV-2 displays a better sensitivity to IFN-I in vitro compared with SARS-CoV-1 in infected cell lines. Therefore, early administration of IFN-α2 might be promising for patients with COVID-19, especially in those who demonstrate a defective IFN response. The timing of IFN exposition may be critical to control the virus and avoid immunopathogenesis. Channappanavar et al. have shown that delayed IFN-I expression can be detrimental in mice in the context of SARS-CoV-1 infection. Our data suggest that screening patients for IFN production is instrumental to select those who could benefit from early intervention with IFN. Following day 10, IL-6 remains increased whereas IFN-α tapered. This kinetics highlight that cytokine inhibitors could be helpful at the second phase of the disease following IFN-I decrease. Viral characteristic or individual genetic susceptibility should be explored to understand the defect of IFN-α production in some patients with COVID. Some IFN-α2–positive patients also experienced fatal outcome, highlighting the multifactorial causes of disease severity. We acknowledge limitations of this study, related to the small number of included patients and the technical limitation for the measurement of IFN-β and IFN-λ, in this proof-of-concept study.

Here, we provide new arguments for an early intervention with recombinant IFN-α2 and we also highlight the window of opportunity for immunosuppressors at the second phase of the disease, opening new avenues in COVID-19 therapies.

7. Kelly EA, Esaatli S, Liu LY, Evans MD, Johansson MW, Mathur S, et al. Mepolizumab attenuates airway eosinophil numbers, but not their functional phenotype, in asthma. Am J Respir Crit Care Med 2017;196:1385-95.
8. Li Y, Zeng Z, Cao Y, Liu Y, Ping F, Liang M, et al. Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF-kappaB signaling pathways. Sci Rep 2016;6:27911.
9. Centers for Disease Control and Prevention. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 - COVID-NET, 14 States, March 1-30, 2020. MMWR 2020;69.

Available online April 22, 2020.
https://doi.org/10.1016/j.jaci.2020.04.009
METHODS

In all 3 studies, total RNA was extracted from epithelial brush samples preserved in RLT buffer (Qiagen, Germantown, Md). Samples were thawed, vortexed, and quick-spun, after which the supernatant was transferred to fresh tubes. The samples were then spun through a Qiashredder column (Qiagen) and extracted by using RNeasy mini kits (Qiagen) with 25-μL elution volumes following the manufacturer’s protocol. In the cat allergy upper airway challenge study, sequencing libraries were constructed from total RNA by using TruSeq RNA Sample Preparation Kits v2 (Illumina). In the URECA and adult asthma studies, sequencing libraries were constructed from total RNA by using SMART-Seq v4 Ultra Low Input RNA Kit (Takara). For each study, libraries were clustered onto a flow cell by using a cBOT amplification system with a HiSeq SR v4 Cluster Kit (Illumina). Single-read sequencing was carried out on a HiSeq2500 sequencer (Illumina), using a HiSeq SBS v4 Kit to generate 58-bp reads, with a target of approximately 10 million reads per sample. Samples for each study were processed and sequenced independently.

Reads were processed by using workflows managed on the Galaxy platform. Reads were trimmed by 1 base at the 3’ end and then trimmed from both ends until base calls had a minimum quality score of at least 30 (Galaxy FASTQ Trimmer tool [version 1.0.1]). FastqMcf (version 1.1.2) was used to remove any remaining adapter sequence. To align the trimmed reads, we used the STAR aligner with the GRCh38 reference genome and gene annotations from ensembl, release 91. Gene counts were generated by using HTSeq-count (version 0.4.1). For quality control, the samples kept were those that had counts greater than 1 million, more than 80% of reads aligned, and a median coefficient of variation (CV) coverage less than 1. Genes were filtered to include those that had a trimmed mean of M-value (TMM) normalization count of at least 1 in at least 10% of libraries and were classified as protein coding by using BioMart. Counts were transformed to log2 counts per million along with observation-level weights by using voom with quality-adjusted weights from the limma R package to create a weighted gene expression matrix suitable for downstream analyses.

Differential expression of \textit{ACE2} was assessed independently in each data set by using a weighted linear mixed effects model (limma) appropriate for RNA sequencing data and an empirical Bayes method. Mixed effects linear regression models were used; they included relevant clinical or technical variables (for URECA, cytologically determined cell percentages in the brush and the clinical site were used; for the upper airway challenge study, processing batch was used; and for the adult asthma study, no fixed effects were included) and a random effect of participant in both of the airway challenge studies. \( P \) values less than .05 were considered statistically significant.

We searched the National Center for Biotechnology Information Gene Expression Omnibus for the terms \textit{IL13} and \textit{epithelial} subset to the organism \textit{Homo sapiens}. From this we identified 2 studies investigating the effects of IL-13 stimulation on human airway epithelial cells grown at an air-liquid interface that had repeated measures in the IL-13 stimulation and unstimulated groups. In the GSE110799 study, human nasal epithelial cells isolated from nasal turbinates were cultured in an air-liquid interface until the full differentiation was complete, after which the differentiated cells at ALI-D47 were incubated with 100 ng/mL of IL-13 for 3 days. In GSE37693, RNA was isolated from primary culture airway epithelial cells grown at an air-liquid interface treated with or without IL-13 for 21 days. Differential expression analysis was performed by using GEO2R, which performs voom and limma in the National Center for Biotechnology Information’s Gene Expression Omnibus browser.

REFERENCES

E1. Smedley D, Haider S, Durinck S, Pandini L, Provero P, Allen J, et al. The BioMart community portal: an innovative alternative to large, centralized data repositories. Nucleic Acids Res 2015;43:W589-98.

E2. Liu R, Holik AZ, Su S, Jansz N, Chen K, Leong HS, et al. Why weight? Modelling sample and observational level variability improves power in RNA-seq analyses. Nucleic Acids Res 2015;43:e97.

E3. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.

E4. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets-update. Nucleic Acids Res 2013;41:D991-5.

E5. Alevy YG, Patel AC, Romero AG, Patel DA, Tucker J, Rowwit WT, et al. IL-13-induced airway mucus production is attenuated by MAPK13 inhibition. J Clin Invest 2012;122:4555-68.
FIG E1. IL-13 stimulation decreases ACE2 expression in nasal and bronchial epithelium. IL-13 stimulation of airway epithelial cells grown in an air-liquid interface decreased ACE2 expression in nasal epithelium (FC = 0.44; P = 5.8E–4; n = 2 per condition) (A) and bronchial epithelium (FC = 0.80; P = 5.1E–3; n = 4 per condition) (B). Shown are mean expression levels (red) and individual points representing biologic replicates.
| Biomarker                        | Association with ACE2 expression ($β$ coefficient) | $P$ value |
|---------------------------------|----------------------------------------------------|-----------|
| Positive allergen-specific IgE  | -0.089                                             | 3.1E−5    |
| Total IgE level                 | -0.31                                              | 5.1E−6    |
| Fractional exhaled nitric oxide | -0.45                                              | 3.4E−3    |
| Blood eosinophils               | -0.13                                              | .07       |
| Nasal epithelial IL-13 expression| -0.123                                             | 8.6E−5    |