Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, ACE2

Daniel J. Jackson, MD, William W. Busse, MD, Leonard B. Bacharier, MD, Meyer Kattan, MD, George T. O’Connor, MD, Robert A. Wood, MD, Cynthia M. Visness, PhD, Stephen R. Durham, MD, David Larson, PhD, Stephane Esnault, PhD, Carole Ober, PhD, Peter J. Gergen, MD, Patrice Becker, MD, Alkis Togias, MD, James E. Gern, MD, Mathew C. Altman, MD

PII: S0091-6749(20)30551-0
DOI: https://doi.org/10.1016/j.jaci.2020.04.009
Reference: YMAI 14507
To appear in: Journal of Allergy and Clinical Immunology

Received Date: 7 April 2020
Revised Date: 15 April 2020
Accepted Date: 16 April 2020


This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.
Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, ACE2

Daniel J. Jackson, MD,1 William W. Busse, MD,1 Leonard B. Bacharier, MD,2 Meyer Kattan, MD,3 George T. O’Connor, MD,4 Robert A. Wood, MD,5 Cynthia M. Visness, PhD,6 Stephen R. Durham, MD,7 David Larson, PhD,8 Stephane Esnault, PhD,2 Carole Ober, PhD,9 Peter J. Gergen, MD,10 Patrice Becker, MD,10 Alkis Togias, MD,10 James E. Gern, MD,1 Mathew C. Altman, MD11,12

1) University of Wisconsin School of Medicine and Public Health
2) Washington University School of Medicine
3) Columbia University College of Physicians and Surgeons
4) Boston University School of Medicine
5) Johns Hopkins University School of Medicine
6) Rho, Inc.
7) MRC and Asthma UK, Centre in Allergic Mechanisms of Asthma
8) The Immune Tolerance Network
9) University of Chicago
10)The National Institute of Allergy and Infectious Disease
11)Department of Medicine, University of Washington
12)Benaroya Research Institute, Systems Immunology Division

Corresponding Author:
Daniel J. Jackson, MD
Associate Professor of Pediatrics and Medicine
University of Wisconsin School of Medicine and Public Health
600 Highland Avenue
CSC K4/936
Phone: 608-263-7686
Fax: 608-265-2207
Email: djj@medicine.wisc.edu

Word Count: 1140

Capsule Summary:
Underlying respiratory allergy and experimental allergen exposure reduce the expression of the SARS-CoV-2 receptor, ACE2, which could lead to reduced COVID-19 susceptibility.

Key Words: SARS-CoV-2, COVID-19, asthma, respiratory allergy, allergic sensitization, receptor, ACE2 expression
Funding:
Funded by NIH/NIAID: UM1AI114271 & UM1AI109565 and NIH/NHLBI: RO1HL12384

Dr. Jackson reports grants from NIH/NIAID/NHLBI and GlaxoSmithKline, personal fees for DSMB from Pfizer and for consulting from Novartis, Sanofi-Regeneron, GlaxoSmithKline, Vifor Pharma and Astra Zeneca.
Dr. Busse reports grants from NIH/NIAID/NHLBI, during the conduct of the study; personal fees from Novartis, Sandoz, Regeneron, AstraZeneca, GlaxoSmithKline, Genentech, Teva, Elsevier, Arrowhead, resTORbio, Med Learning Group, Practicing Clinicians Exchange, Boston Scientific, Medscape.
Dr. Bacharier reports grant support from NIH/NIAID/NHLBI, Sanofi and Vectura, and personal fees from GlaxoSmithKline, Genentech, Novartis, Merck, DBV Technologies, Teva, Boehringer Ingelheim, AstraZeneca, WebMD/Medscape, Sanofi, Regeneron, Vectura, and Circassia.
Dr. Wood receives grant support from the NIH, Astellas, Aimmune, DBV, Sanofi, and Regeneron, and royalties from Up To Date.
Dr. Gern reports grants from NIH, personal fees and stock options from Meissa Vaccines Inc., personal fees from AstraZeneca and Ena Therapeutics and a patent on methods for production of rhinoviruses.
Dr. Altman reports personal fees for consulting from Regeneron.
Drs. Becker, Durham, Esnault, Gergen, Kattan, Larson, Ober, O’Connor, Togias, and Visness report no conflicts of interest.

Conflict of Interest

Dr. Jackson reports grants from NIH/NIAID/NHLBI and GlaxoSmithKline, personal fees for DSMB from Pfizer and for consulting from Novartis, Sanofi-Regeneron, GlaxoSmithKline, Vifor Pharma and Astra Zeneca.
Dr. Busse reports grants from NIH/NIAID/NHLBI, during the conduct of the study; personal fees from Novartis, Sandoz, Regeneron, AstraZeneca, GlaxoSmithKline, Genentech, Teva, Elsevier, Arrowhead, resTORbio, Med Learning Group, Practicing Clinicians Exchange, Boston Scientific, Medscape.
Dr. Bacharier reports grant support from NIH/NIAID/NHLBI, Sanofi and Vectura, and personal fees from GlaxoSmithKline, Genentech, Novartis, Merck, DBV Technologies, Teva, Boehringer Ingelheim, AstraZeneca, WebMD/Medscape, Sanofi, Regeneron, Vectura, and Circassia.
Dr. Wood receives grant support from the NIH, Astellas, Aimmune, DBV, Sanofi, and Regeneron, and royalties from Up To Date.
Dr. Gern reports grants from NIH, personal fees and stock options from Meissa Vaccines Inc., personal fees from AstraZeneca and Ena Therapeutics and a patent on methods for production of rhinoviruses.
Dr. Altman reports personal fees for consulting from Regeneron.
Drs. Becker, Durham, Esnault, Gergen, Kattan, Larson, Ober, O’Connor, Togias, and Visness report no conflicts of interest.
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 COPD. The Center for Disease Control (CDC) 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 COVID-19 illness severity.

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

We hypothesized that one 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 three different cohorts of children and adults. In all three 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 online supplement.(5) Differential expression of ACE2 was assessed using a weighted linear mixed effects model (limma) appropriate for RNA-seq data and empirical Bayes method.

Children at high risk for asthma based upon parental histories and living in urban neighborhoods were enrolled prenatally and followed prospectively in the Urban Environment and Childhood Asthma (URECA) cohort and 318 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/minimal, low, medium, and high] as previously described.(6) Additional type 2 biomarkers, including fractional exhaled nitric oxide (FeNO), peripheral blood eosinophils, and total IgE, were evaluated using standard methods. In URECA, allergic sensitization was inversely related to ACE2 expression in nasal epithelium regardless of asthma status (Figure 1A). Within children with asthma, moderate allergic sensitization (fold change (FC)=0.70, p=4.2E-3) and high allergic sensitization (FC=0.54, p=6.4E-5) were associated with progressively greater reductions in ACE2 compared to children with asthma but no/minimal allergic sensitization (Figure 1B). ACE2 expression was also significantly inversely associated with type 2 biomarkers (Supplementary Table 1)
including the number of positive allergen-specific IgE tests (beta coefficient -0.089, p=3.1E-5), total IgE (beta coefficient -0.31, p=5.1E-6), FeNO (beta coefficient -0.45, p=3.4E-3), and nasal epithelial IL13 expression (beta coefficient -0.123, p=8.6E-5). ACE2 expression was not significantly correlated with peripheral blood eosinophils (beta coefficient -0.13, p=0.07). Although male sex has been associated with increased COVID-19 illness severity(2), 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, without asthma symptoms in the prior year, who were enrolled in a study where they underwent nasal cat allergen challenge (NAC) and exposure to cat allergen through an environmental exposure chamber (EEC) as previously described.(5) Pre/post-allergen challenge nasal brush samples were obtained. Allergen exposure by both NAC and EEC led to significant reductions in ACE2 expression (Figure 2A; NAC: FC=0.81, p=2.4E-3; EEC: FC=0.79, p=1.6E-3).

An additional cohort of 23 adult participants with mild asthma, not treated with asthma controller therapy, underwent segmental allergen bronchoprovocation to dust mite, ragweed, or cat, as previously described.(7) Pre/post-allergen challenge bronchial brushings were obtained and demonstrated significantly reduced ACE2 expression in lower airway epithelium post-allergen challenge (Figure 2B: FC 0.64, p=0.01).

From in vitro models obtained from 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 (Supplemental Figure 1) in both nasal (FC=0.44 p=5.8E-4) and bronchial epithelium (FC=0.80, p=5.1E-3).

Viral respiratory infections are the most common trigger of severe asthma exacerbations in children and adults. Unexpectedly, large epidemiological studies of the COVID-19 pandemic in China did not identify asthma as a risk factor of severe COVID-19 related illnesses.(2) 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, non-atopic 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 allergic individuals, and elucidation of these factors may also provide important insights into COVID-19 disease pathogenesis.

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. 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, though previous work suggests a direct association between ACE2 mRNA levels and ACE2 protein levels in the lung.(8)
It is important to note that early data in the US suggest a higher rate of asthma in patients hospitalized for severe COVID-19 illness, but do not specify whether asthma was allergic or not, an important differentiation that relates to our findings, nor the potential presence of other co-morbidities, 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, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
William W. Busse, MD, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
Leonard B. Bacharier, MD, Washington University School of Medicine, St. Louis, MO, USA
Meyer Kattan, MD, Columbia University College of Physicians and Surgeons, New York, NY, USA
George T. O’Connor, MD, Boston University School of Medicine, Boston, MA, USA
Robert A. Wood, MD, Johns Hopkins School of Medicine, Baltimore, MD, USA
Cynthia M. Visness, PhD, Rho, Inc., Durham, NC, USA
Stephen R. Durham, MD, MRC and Asthma UK, Centre in Allergic Mechanisms of Asthma, London, UK
David Larson, PhD, The Immune Tolerance Network, Bethesda, MD, USA
Stephane Esnault, PhD, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
Carole Ober, PhD, University of Chicago, Chicago, IL, USA
Peter J. Gergen, MD, The National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
Patrice Becker, MD, The National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
Alkis Togias, MD, The National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
James E. Gern, MD, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
Mathew C. Altman, MD, University of Washington and Benaroya Research Institute, Seattle, WA, USA
References


Figure Legends

Figure 1. ACE2 expression is decreased in the nasal epithelium of children with allergic sensitization and allergic asthma.
(A) ACE2 expression levels in nasal brush samples from 11 year old children in the URECA cohort according to asthma diagnosis by age 10, dichotomized as No(-) or Yes(+), and IgE sensitization trajectory at age 10, dichotomized as not/minimally IgE sensitized (-) or IgE sensitized (+), showing lower levels of ACE2 in children with atopy and atopic asthma. (B) ACE2 expression in URECA children with asthma, subdivided according to the degree of IgE sensitization, demonstrating progressively lower levels of ACE2 according to the degree of IgE sensitization among children with asthma. Children with both asthma and the highest IgE sensitization had the lowest levels of ACE2 expression. Expression levels are log2 transformed, shown are median values (horizontal), interquartile ranges (boxes), and 1.5 IQR (whiskers). The printed fold...
changes (FC) are for the non-log2-transformed expression values to aid in interpretation of the effect sizes.

Figure 2. ACE2 expression is decreased in nasal and bronchial epithelium of allergic individuals after allergen challenge. 
(A) ACE2 expression was significantly decreased in nasal brush samples from adults in the cohort with allergic rhinitis and cat allergen sensitization both 8 hours after a cat allergen NAC, and 8 hours after the second day of a cat allergen EEC (n=24). (B) ACE2 was significantly decreased in bronchial epithelial brush samples from adults with allergic asthma 48 hours after a segmental bronchial allergen challenge (n=23).
Expression levels are log2 transformed, shown are median values (horizontal), interquartile ranges (boxes), and 1.5 IQR (whiskers). The printed fold changes (FC) are for the non-log2-transformed expression values to aid in interpretation of the effect sizes.
Figure 1

A

ACE2 Expression (log2)

Asthma / Atopy Grouping

B

Degree of Sensitization within Asthma

ANOVA p=1.5E-6

ANOVA p=3.5E-6

FC=0.76, p=1.0E-4

FC=0.67, p=3.3E-6

FC=0.88, p=0.13

FC=0.70, p=4.2E-3

FC=0.54, p=6.4E-5

n=106

n=117

n=34

n=61

n=34

n=7

n=36

n=18

(-) Asthma

(+Asthma

(-) IgE Sens

(+) IgE Sens

(-) Asthma

(+Asthma

(-) IgE Sens

(+) IgE Sens

(+Asthma

(-) IgE Sens

(+Asthma

(-) IgE Sens

No/IgE Sens

Low IgE Sens

Medium IgE Sens

High IgE Sens
Figure 2

A

ACE2 Expression (log2)

Baseline | Post MAC | Post EEC

FC=0.81, p=2.4E-3

FC=0.79, p=1.6E-3

B

Baseline | Post bronchial allergen challenge

FC=0.64, p=0.01

Sample Collection Timepoint
Supplementary Methods:

In all three studies, total RNA was extracted from epithelial brush samples preserved in RLT buffer (Qiagen, MD, USA). Samples were thawed, vortexed, and quick-spun, and the supernatant transferred to fresh tubes. The samples were then spun through a QiaShredder column (Qiagen) and extracted 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 using TruSeq RNA Sample Preparation Kits v2 (Illumina). In the URECA and adult asthma studies, sequencing libraries were constructed from total RNA using SMART-Seq v4 Ultra Low Input RNA Kit (Takara). For each study, libraries were clustered onto a flowcell 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-base reads, with a target of approximately 10 million reads per sample. Sample for each study was processed and sequenced independently.

Reads were processed 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 v1.0.0). FastqMcf (v1.1.2) 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 using HTSeq-count (v0.4.1). For quality control, samples were kept that had counts >1 million, percent of reads aligned >80% and median CV coverage <1. Genes were filtered to include those that had a trimmed mean of M values (TMM) normalization count of at least 1 in at least 10% of libraries and were classified as protein coding using BioMart(1). Counts were transformed to log2 counts per million along with observations level weights using voomWithQualityWeights from the limma R package(2) to create a weighted gene expression matrix suitable for downstream analyses.

Differential expression of ACE2 was assessed independently in each dataset using a weighted linear mixed effects model (limma) appropriate for RNA-seq data and empirical Bayes method(2, 3). Mixed-effects linear regression models were used including relevant clinical or technical variables (for URECA, cytologically determined cell percentages in the brush and the clinical site; for the upper airway challenge study, processing batch; 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 <0.05 were considered statistically significant.

We searched NCBI’s Gene Expression Omnibus for the terms “IL13” and “epithelial” subset to organism homo sapiens.(4) From this we identified two studies investigating the effects of IL-13 stimulation on human airway epithelial cells grown at air liquid interface that had repeated measures in the IL-13 stimulation and unstimulated groups. GSE110799 has the study design: “Human nasal epithelial cells isolated from nasal turbinates were cultured in air-liquid interface (ALI) until the full differentiation was complete. Differentiated cells at ALI-D47 were incubated with 100 ng/mL of IL-13 for 3
days.” GSE37693 has the study design: “RNA was isolated from primary culture airway epithelial cells grown at air-liquid interface, treated with or without IL-13 for 21 days”.(5) Differential expression analysis was performed using GEO2R, which performs voom and limma(2, 3) in the NCBI GEO browser.
**Supplementary Table 1**: Association of T2 biomarkers & nasal brush ACE2 Expression in the URECA cohort.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Association with ACE2 Expression (β coefficient)</th>
<th>(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td># of positive allergen-specific IgE</td>
<td>-0.089</td>
<td>3.1E-5</td>
</tr>
<tr>
<td>Total IgE</td>
<td>-0.31</td>
<td>5.1E-6</td>
</tr>
<tr>
<td>Fractional exhaled nitric oxide (FeNO)</td>
<td>-0.45</td>
<td>3.4E-3</td>
</tr>
<tr>
<td>Blood eosinophils</td>
<td>-0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Nasal epithelial IL13 expression</td>
<td>-0.123</td>
<td>8.6E-5</td>
</tr>
</tbody>
</table>
Supplementary Figure 1. 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 (A) nasal epithelium (FC=0.44, p-value=5.8E-4; n=2 per condition) and (B) bronchial epithelium (FC=0.80, p-value=5.1E-3; n=4 per condition). Shown are mean expression levels (red) and individual points representing biological replicates.
Supplementary References


Supplementary Figure 1

Nasal Epithelium

Bronchial Epithelium

Sample Treatment