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Letter

SARS-CoV-2 infection in severe asthma is associated with worsening of COVID-19 through respiratory NLRP3 inflammasome activation

To the Editor,

Recent clinical data suggest that the outcome of coronavirus disease 2019 (COVID-19) in asthmatic patients depends on the subtype and severity of asthma (1,2); however, there is limited information on the pathobiological outcomes of superimposed SARS-CoV-2 infection in asthma, especially in severe disease.

To investigate the effects of SARS-CoV-2 infection on severe asthma, we developed a novel mouse model using SARS-CoV-2-susceptible hACE2 transgenic mice, K18-hACE2 (3). Specifically, we first established *Aspergillus fumigatus* (*Af*)-induced severe eosinophilic allergic lung inflammation using fungal extracts (4), and challenged the mice intranasally with SARS-CoV-2 on the same day as the last *Af* challenge (Figure 1A).

Forty-eight hours after the last challenge with *Af*, when fungus-induced eosinophilic airway inflammation predominated, greater infiltration of inflammatory cells was noted in the peribronchiolar and perivascular areas in the *Af*-challenged mice after SARS-CoV-2 infection. This was supported by the increased number of eosinophils in the bronchoalveolar lavage fluid (BALF) (Figure 1B,C). Moreover, in parallel with the increase in neutrophils in BALF, SARS-CoV-2 infection led to pronounced infiltration of alveolar inflammatory cells in *Af*-challenged mice compared with those sensitized only to *Af* and infected with the virus.

Cytokines implicated in severe asthma after SARS-CoV-2 infection were significantly increased in the lung tissues of *Af*-challenged mice (Figure 1D). In mice sensitized only to *Af*, SARS-CoV-2 infection did not induce hyperinflammatory changes in the lungs at 48 h, when they were in the early stages of infection (3). However, the pulmonary levels of the hyperinflammation markers of COVID-19 were dramatically increased in *Af*-challenged mice 48 h after viral infection (Figure 1E). Experiments using the delta variant B.1.617.2 [NCCP43390] showed similar findings (Figure 1F,G).

The NLRP3 inflammasome was recently reported to play a crucial role in the pathobiology of COVID-19 (5). We analyzed six public datasets of gene expression profiles of airway and blood specimens from COVID-19 patients and found that the expression of NLRP3 inflammasome-related genes was significantly higher in airway specimens than in blood samples (Figure S1A,B). Furthermore, by analyzing a single-cell RNA sequencing dataset (6), we characterized several clusters of macrophages

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and dendritic cells harboring activated NLRP3 inflammasome in nasal swab samples from patients with early COVID-19 (Figure S1C,D).

Given the crucial role of the NLRP3 inflammasome in the pathogenesis of current *Af*-induced severe asthma (4), we investigated whether pre-existing asthmatic inflammation influenced SARS-CoV-2-induced NLRP3 inflammasome activation in the lungs of mice. Notably, significant increases in NLRP3 inflammasome components (NLRP3, ASC, cleaved caspase-1) and mature IL-1β were observed after SARS-CoV-2 infection in *Af*-challenged mice compared with *Af*-challenged mice that had not been infected with SARS-CoV-2 or SARS-CoV-2-infected mice that had not been challenged with *Af* (Figure 2A,B). As expected, blockade of IL-1β remarkably reduced SARS-CoV-2-induced pulmonary inflammation in these mice (Figure 2C), implying that SARS-CoV-2 infection in severe asthma is associated with worsening of COVID-19 and asthmatic inflammation through NLRP3 inflammasome activation. Transcriptome analyses of the lung tissue from mice and a transcriptome-based predicted gene interaction network analysis further validated these findings (Figure 2D,E). In a population-based large nationwide cohort, we also observed a higher severity of COVID-19 in asthma patients requiring systemic corticosteroid administration (Figure S2).

In conclusion, SARS-CoV-2 infection in severe asthma may be associated with worsening of COVID-19 through respiratory NLRP3 inflammasome activation. Targeting the NLRP3 inflammasome may be a promising approach for early treatment of COVID-19 in a specific molecular phenotype of severe asthma including fungus-induced severe eosinophilic allergic subtype associated with a dysregulated innate immune response.

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Author contributions

JSJ interpreted the data and wrote the manuscript. JYC, SOP, HJP, and KHP conducted the experiments and performed the analysis. WK and YY performed transcriptomic analyses. JSJ, JYC, JSK, SOP, WK, and YY generated the figures. JSK and DHK conducted analysis of the large-scale national COVID-19 cohort. JK and GYK performed single-cell RNA sequencing analysis and data interpretation. JSJ, SKE, and YCL designed the study and directed the project.

Conflict of Interest

The authors have no conflicts of interest in relation to this work.

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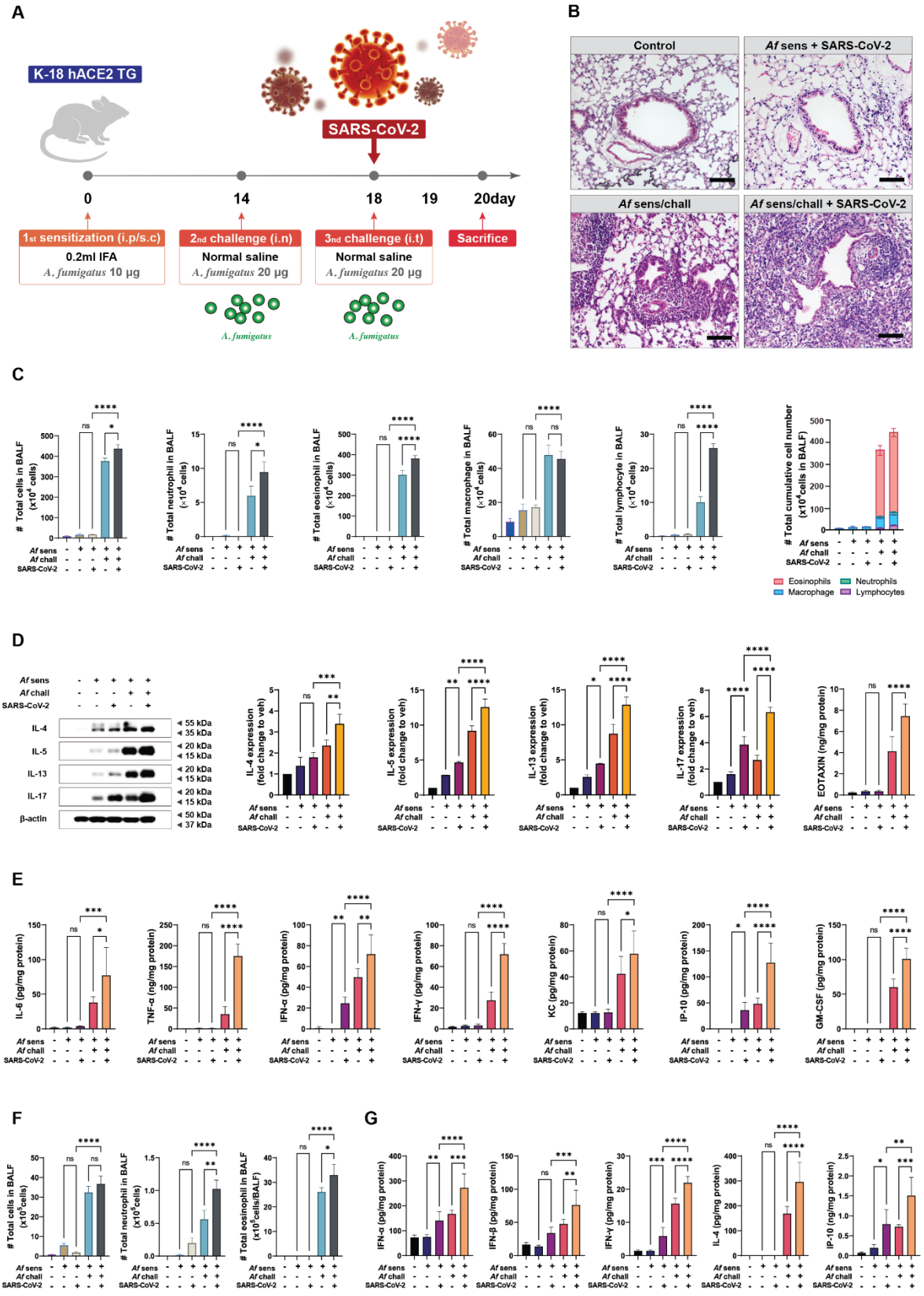

Figure 1 (A) Experimental design for SARS-CoV-2 infection in *Aspergillus fumigatus* (*Af*)-induced severe asthma. (B) Hematoxylin and eosin (H&E)-stained lung tissue

sections. Scale bars, 100 μm. (C) Cells in bronchoalveolar lavage fluid (BALF). (D) IL-4, IL-5, IL-13, IL-17, and eotaxin levels in the lungs. (E) IL-6, TNF-α, IFN-α, IFN-γ, KC, IP-10, and GM-CSF levels in the lungs. (F, G) BALF cells (F) and IFNs, IL-4, and IP-10 in the lungs of *Af*-challenged mice infected with the delta variant (G). Data are means ± SEM from at least two experiments (six mice per group for each experiment). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001.

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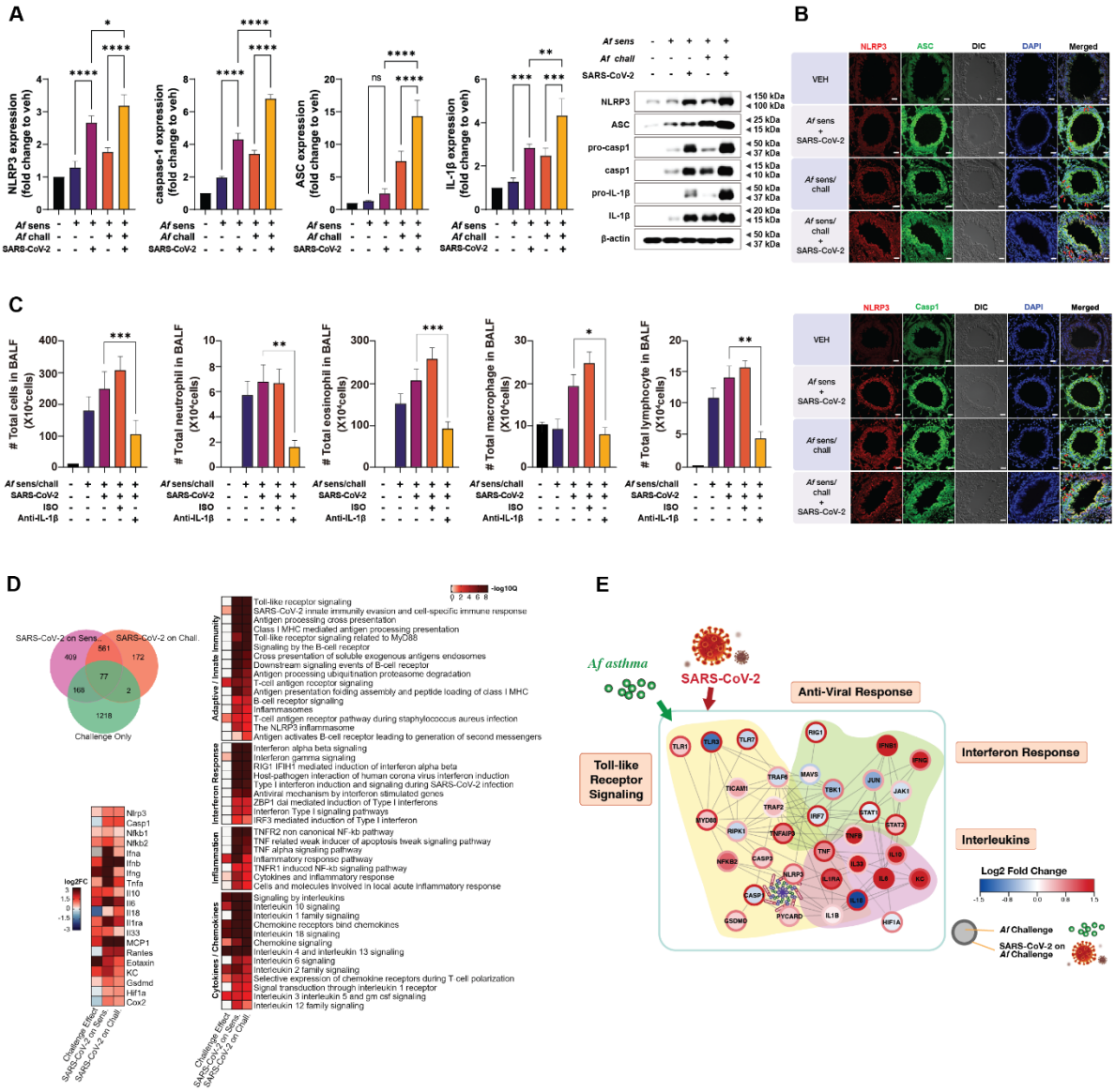

Figure 2 (A) NLRP3, caspase-1 (casp1), ASC, and IL-1β in the lungs. (B) Confocal images of lung tissue showing NLRP3, Casp1, and ASC expression. (C) Cells in bronchoalveolar lavage fluid. ISO, isotype antibody; Anti-IL-1β, anti-IL-1β neutralizing antibody. (D) Transcriptome analysis of lung tissue. (E) Interaction network of genes involved in toll-like receptor signaling, antiviral response, and immune and inflammatory cytokine pathways. Data are presented as means ± SEM of the levels derived from at least two experiments (six mice per group for each experiment). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001.

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