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; 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. Specifically, we first established Aspergillus fumigatus (Af)-induced severe eosinophilic allergic lung inflammation using fungal extracts 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. 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. 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, we characterized several clusters of macrophages 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, 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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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
This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2020R1A2C2101942; Y.C.L.), by grants from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant numbers: HI20C1933 and HI22C1124), by Digital Healthcare Research Grant through the Seokchun Caritas Foundation (SCY2113P) and by the fund of Biomedical Research Institute, Jeonbuk National University Hospital.
CONFLICT OF INTEREST
The authors have no conflicts of interest in relation to this work.

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