Cellular & Molecular Immunology www.nature.com/cmi

CORRESPONDENCE

Pyroptotic macrophages stimulate the SARS-CoV-2-associated cytokine storm

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Cellular & Molecular Immunology (2021) 18:1305–1307; https://doi.org/10.1038/s41423-021-00665-0

Coronavirus disease 2019 (COVID-19) is an unprecedented pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 22 February 2021, the worldwide pandemic has resulted in more than 110 million cases and 2.4 million deaths.[1] Clinical investigation of COVID-19 patients has shown that a systemic cytokine storm can occur, especially in severe cases.[2] Treatment of the SARS-CoV-2-associated cytokine storm with tocilizumab[3] or anakinra[4] has been shown to immediately improve the clinical outcome in most severe and critical COVID-19 patients. These data highlight the systemic cytokine storm as an important exacerbating event in severe COVID-19; however, our understanding of the molecular mechanisms involved in the initiation of the SARS-CoV-2-associated cytokine storm is limited. In the present study, we uncovered a reasonable explanation for cytokine storm initiation through the analysis of 13 autopsy samples from severe COVID-19 patients.

To investigate SARS-CoV-2-associated cytokine storm processes, we collected three control lung tissues from patients without SARS-CoV-2 infection as a control group and six lung tissues from COVID-19 patients as a patient group for RNA-seq transcriptome analysis. The clinical data of the patients are presented in the Supplementary material (Table S1). Principal component analysis (PCA) of the RNA-seq data showed clear biological differences between the control and patient group transcriptomes (Fig. S1a, b). Through differential gene expression analysis, we identified 1951 upregulated genes and 1971 downregulated genes in the patient group compared with the control group (Fig. S1c). Functional analysis of the upregulated genes revealed enrichment in functions related to ROS activation, virus infection-related signaling pathways, the HIF-1α signaling pathway, the NOD-like receptor signaling pathway, and metabolic dysregulation (Fig. S1d–f). Previous studies have suggested that cellular "danger" signals, including infection, ROS, and metabolic dysregulation, could trigger the NLRP3 inflammasome signaling pathway.[5] The data presented herein demonstrate that many cellular "danger" signals are activated in the pulmonary microenvironment of severe COVID-19 patients and indicate that NLRP3 inflammasome signaling is involved in the pathogenesis of COVID-19.

Notably, we found that chemokines responsible for recruiting monocytes were significantly upregulated in the lung tissues of COVID-19 patients (Fig. S1g), suggesting that SARS-CoV-2 infection may lead to the recruitment of monocytes to the lungs of patients. To further test this hypothesis, we used the xCell method to analyze immune infiltration in our samples. We found significantly more infiltration of monocytes, neutrophils, and plasma cells in the lung tissues of severe COVID-19 patients than in the tissues of control donors. There were no significant changes in the levels of other immune cells (Fig. 1a). Immunohistochemical analysis also revealed that many $CD14^+CD16^+$ double-positive proinflammatory monocytes infiltrated the alveoli of severe COVID-19 patients (Fig. 1b), many of which transformed into CD163-positive macrophages (Fig. 1c). Notably, CD163 staining in infiltrating macrophages was more evident in COVID-19 patients than in controls, indicating that these infiltrating proinflammatory macrophages underwent activation switching.[6] We also observed the infiltration of some $CD4^+$ and $CD8^+$ T cells into the alveoli of COVID-19 patients (Fig. S2a, b).

We previously reported that proinflammatory monocytes play a crucial role in the SARS-CoV-2-associated cytokine storm.[7] To determine whether these infiltrating proinflammatory macrophages are the initiators of the SARS-CoV-2-associated cytokine storm, we measured the levels of several key proinflammatory cytokines associated with the clinical deterioration of COVID-19 patients, namely, IL-1β, IL-6, and IL-18, in the lung tissues of severe COVID-19 patients and control donors. As expected, the expression of these proinflammatory cytokines was higher in COVID-19 patients than in control donors, suggesting that the cytokine storm originates in the lungs of patients (Fig. S3a–c). Furthermore, costaining with CD163 revealed that IL-1β, IL-6, and IL-18 were more highly expressed in the pulmonary macrophages of COVID-19 patients than in those of control donors, suggesting that proinflammatory macrophages are involved in the SARS-CoV-2-associated cytokine storm (Fig. 1d).

In addition, we found that the infiltrating macrophages had characteristics of edema, which is a morphological feature of pyroptosis[8] (Fig. 1e). Therefore, we propose that pyroptosis may be a possible mechanism underlying the cytokine storm in severe COVID-19 patients. Gasdermin D (GSDMD) is a pore-forming protein and a well-known trigger of pyroptosis. To further confirm the role of pyroptosis in pulmonary proinflammatory macrophages after SARS-CoV-2 infection, we measured the levels of cleaved GSDMD in lung tissues from COVID-19 patients and

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Received: 4 February 2021 Accepted: 24 February 2021
Published online: 19 March 2021

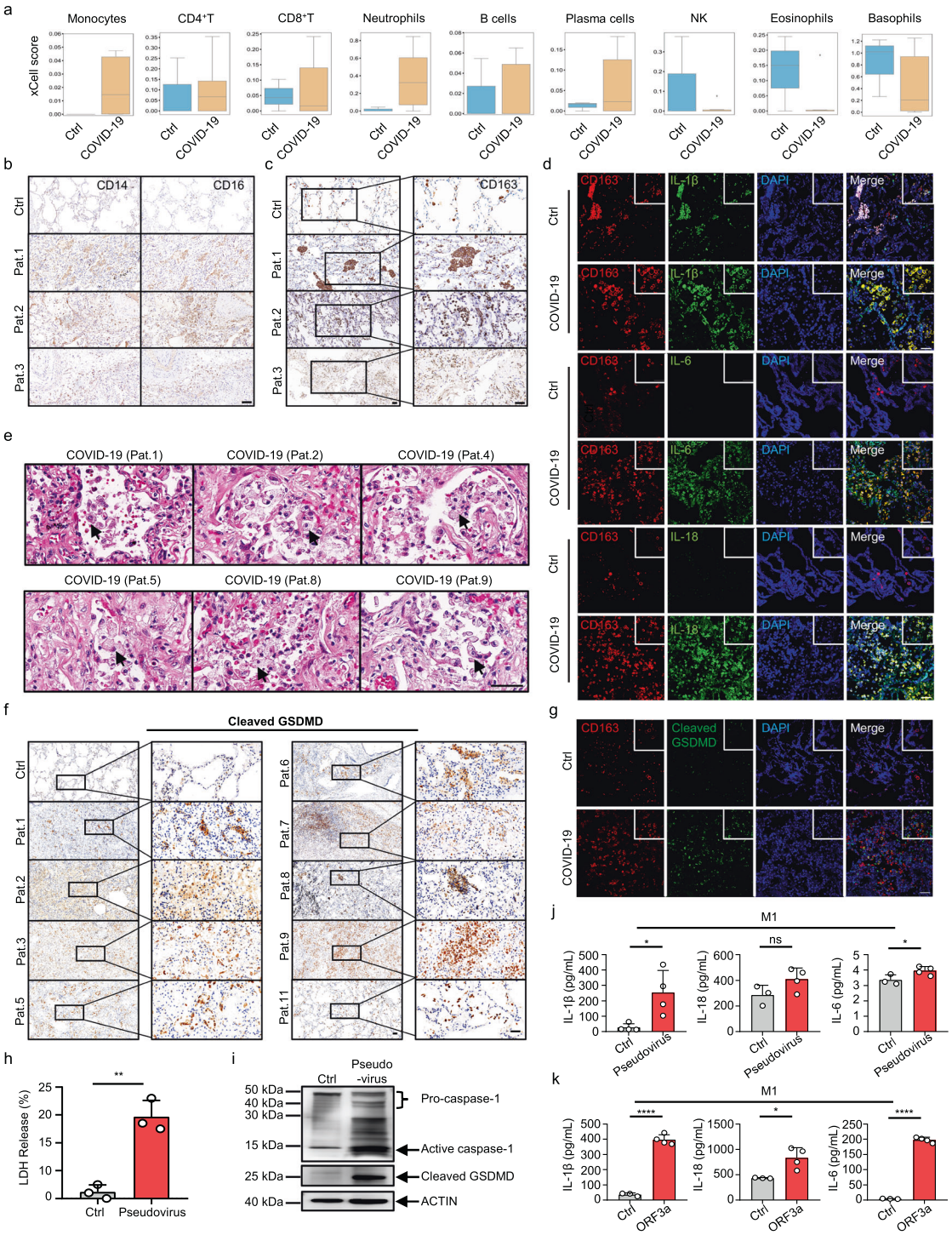

Fig. 1 SARS-CoV-2 promotes cytokine storms by inducing pyroptosis in proinflammatory macrophages in severe COVID-19. **a** Analysis of immune infiltration in the lung samples of control donors and COVID-19 patients using xCell. **b** Representative images of immunohistochemical staining of CD14 and CD16 in the lung samples of control donors and COVID-19 patients (bar = 50 μm). **c** Representative images of immunohistochemical staining of CD163 in the lung tissues of control donors and COVID-19 patients. The right panels (bar = 50 μm) are enlarged versions of the boxed areas in the left panels (bar = 50 μm). **d** Representative confocal microscopy images showing the expression of CD163 (red), IL-1β, IL-6, and IL-18 (green), and the cell nuclei (blue) in the lung tissues of control donors and COVID-19 patients (bar = 50 μm). Enlarged images are embedded in the upper right corner (bar = 10 μm). **e** Hematoxylin and eosin staining of lung tissues from six severe COVID-19 patients (bar = 50 μm). **f** Representative images of immunohistochemical staining of cleaved GSDMD in the lung tissues of control donors and COVID-19 patients. The right panels (bar = 50 μm) are enlarged versions of the boxed areas in the left panels (bar = 50 μm). **g** Representative confocal microscopy images showing the expression of CD163 (red) and cleaved GSDMD (green) and the cell nuclei (blue) in the lung tissue samples of control donors and COVID-19 patients. **h** The amount of lactate dehydrogenase (LDH) released into the culture supernatants of M0 macrophages treated with the SARS-CoV-2 pseudovirus or untreated cells (Ctrl). **i** Western blot analysis of caspase-1, cleaved GSDMD, and ACTIN in M0 macrophages incubated in the presence of SARS-CoV-2 pseudovirus for 12 h. **j**, **k** The levels of IL-1β, IL-6, and IL-18 in cell culture supernatants of M1 macrophages incubated in the presence of SARS-CoV-2 pseudovirus or ORF3a lentivirus for 12 h were measured by ELISA

control donors. Immunohistochemistry revealed that the levels of cleaved GSDMD were significantly higher in the lung tissues of severe COVID-19 patients than in the tissues of control donors, demonstrating exacerbated pyroptosis in the lung tissues of COVID-19 patients (Fig. 1f). Furthermore, multiplex immunohistochemistry confirmed that macrophages were the major cells that were positive for cleaved GSDMD in the lungs (Fig. 1g). Therefore, we hypothesize that pyroptotic macrophages are involved in the SARS-CoV-2-associated cytokine storm.

To test this hypothesis, we examined whether SARS-CoV-2 could activate GSDMD-mediated pyroptosis in macrophages. To this end, we performed a series of in vitro experiments. After induction with PMA, THP-1 cell-derived M0 macrophages were infected with SARS-CoV-2 pseudovirus. We observed that M0 macrophages could indeed be infected by SARS-CoV-2 pseudovirus (Fig. S3d), which triggered a significant upregulation in the release of LDH, IL-1β, and IL-18, which are signature pyroptosis factors (Figs. 1h and S3e). Moreover, active caspase-1 and cleaved GSDMD bands were also observed in SARS-CoV-2 pseudovirus-infected M0 macrophages by Western blot analysis. These data demonstrate that SARS-CoV-2 can induce GSDMD-mediated pyroptosis in macrophages (Fig. 1i). Our previous study showed that interferon-γ (IFN-γ) was a key pathogenic cytokine in COVID-19.[7] Therefore, we treated M0 macrophages with IFN-γ to convert these cells into M1 macrophages and then treated the derived M1 macrophages with SARS-CoV-2 pseudovirus. As expected, the levels of proinflammatory cytokines in the supernatant of M1 macrophages were higher than those in M0 macrophage supernatant (Figs. 1j and S3e).

Chen et al. reported that the SARS-CoV 3a (ORF3a) protein could activate pyroptosis in macrophages.[9] To explore whether the ORF3a protein of SARS-CoV-2 could trigger cytokine storms, we treated macrophages with ORF3a lentivirus. The ORF3a protein not only stimulated macrophages to express IL-1β and IL-18 but also promoted IL-6 production (Figs. 1k and S3f). These data indicated that SARS-CoV-2 could induce macrophages to produce a series of proinflammatory cytokines through pyroptosis.

Collectively, our results confirm the increased numbers of proinflammatory macrophages and the occurrence of GSDMD-mediated pyroptosis in the lung tissues of severe COVID-19 patients, which causes the rapid release of proinflammatory cytokines and cytokine storms. Accordingly, the pyroptosis-associated pathway is a potential therapeutic target to lessen the cytokine storm, especially in severe COVID-19 patients.

ACKNOWLEDGEMENTS

We would like to thank the patients and their families for their selfless donations. We also thank the Huoshenshan Hospital of Wuhan and the General Hospital of Central Theater Command of Wuhan for their permission to carry out the autopsies and to use the samples in this study.

AUTHOR CONTRIBUTIONS

J.Z. designed and performed the experiments, analyzed and interpreted the data, and wrote the manuscript. H.W. designed and performed the experiments and analyzed and interpreted the data. Y.Z. and B.F. assisted with data interpretation and wrote the manuscript with J.Z. X.Y., D.Z., W.W., H.L., Z.W., Z.H., R.S., and Y.R. collected tissue samples and patient information. X.B. designed the experiments, assisted with data interpretation, and collected tissue samples and patient information. Z.T. designed the experiments, assisted with data interpretation. H.W. supervised the project, provided crucial ideas, assisted with data interpretation, and wrote the manuscript.

FUNDING

This work was supported by the China National Center for Biotechnology Development (2020YFC0843800 and 2020YFC0846800), the Natural Science Foundation of China (81922028), and the Youth Innovation Promotion Association of Chinese Academy of Sciences (2019442).

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41423-021-00665-0.

Competing interests: The authors declare no competing interests.

REFERENCES

1. W.H.O. Coronavirus disease (COVID-2019) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (2020).
2. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. **395**, 497–506 (2020).
3. Xu, X. et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc. Natl Acad. Sci. USA* **117**, 10970–10975 (2020).
4. Cavalli, G. et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol.* **2**, e325–e331 (2020).
5. Zhou, R. et al. A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**, 221–225 (2011).
6. Etzerodt, A. & Moestrup, S. K. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxid. Redox Signal* **18**, 2352–2363 (2013).
7. Zhou, Y. et al. Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. *Natl Sci. Rev.* **7**(No. 6), 998–10001 (2020).
8. Shi, J. et al. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem. Sci.* **42**, 245–254 (2017).
9. Chen, I. et al. Severe acute respiratory syndrome coronavirus Viroporin 3a activates the NLRP3 inflammasome. *Front. Microbiol.* **10**, 50 (2019).