

RESEARCH LETTER

Open Access



Anti-influenza T cells in bronchoalveolar microenvironment of critically severe COVID-19 patients

Ming Zheng^{1,2*}

Until December 13, 2021, there have been 269,468,311 confirmed infection cases and 5,304,248 deaths attributed to COVID-19 caused by SARS-CoV-2 virus (<https://covid19.who.int/>). Recently, the global COVID-19 pandemic is merging with the influenza season, leading to an increased risk of COVID-19 and influenza co-infection. The rates of COVID-19 and influenza co-infection were reported to range from 0.2 to 45.7% in different COVID-19 cohorts [1]. Moreover, both SARS-CoV-2 and influenza viruses preferentially infect alveolar type 2 (AT2) cells [2]. The co-infection with influenza A virus causes more severe and prolonged pneumonia in SARS-CoV-2-infected hamsters [3]. However, there is still a lack of evidence to support the co-infection of SARS-CoV-2 with influenza virus in the bronchoalveolar microenvironment of human lungs, and T cell response under this circumstance remains unknown.

The specificity of T cell response is presented by the antigen-specific T cell clonotypes defined by their unique T cell receptors (TCRs) [4]. TCR directly recognizes and interacts with the antigenic epitope, which determines the specificity of T cell response [4]. Thus, the antigenic T cell response can be directly analyzed by epitope-specific TCR sequence [4]. Recently, T-Detect™ COVID (Adaptive Biotechnologies)—the first assay of detecting TCR-β chain—has been approved by the US Food and Drug Administration (FDA) to determine SARS-CoV-2

infection status based on SARS-CoV-2 specific TCR-β sequence [5].

Here, we constructed the analysis framework of digitalized sorting of epitope-specific T cells (DSET; Fig. 1A) according to the amino acid sequence of complementarity determining region 3 (CDR3) in TCR-β, as reported in our previous study [4]. The marker of influenza infection—TCR-β CDR3 sequences of influenza A-specific T cell clonotypes—was collected from previous studies (Fig. 1A). Next, through applying DSET analysis on the single-cell T cell receptor sequencing (scTCR-seq) data of bronchoalveolar lavage fluid (BALF) in a COVID-19 cohort [6], we directly investigated the anti-influenza T cells in bronchoalveolar microenvironment of COVID-19 patients.

We found that 33.3% of COVID-19 patients had positive anti-influenza T cells in BALF (red group; Fig. 1B). Notably, all patients with positive anti-influenza T cells were patients with critical severity. Comparatively, critical infection was observed in only 33.2% patients with negative anti-influenza T cells (blue group; Fig. 1B). Next, we analyzed the abundance of anti-influenza T cells in BALF from COVID-19 patients and its relationship with disease severity defined by mild, severe, and critical COVID-19 infections. The anti-influenza T cells were defined by different specificities to different epitopes from different genes of the influenza A virus. As shown in Fig. 1C, no anti-influenza T cells could be found in COVID-19 patients with mild and severe infections. Comparatively, patients with critical infection had positive anti-influenza T cells with an average percentage of 1.23% (Fig. 1C). Moreover, it is worth noting that two

*Correspondence: mmzheng@fmmu.edu.cn; zhengming_china@163.com

¹ Institute of Military Cognition and Brain Sciences, Academy of Military

Medical Sciences, 27 Taiping Road, Beijing 100850, China

Full list of author information is available at the end of the article

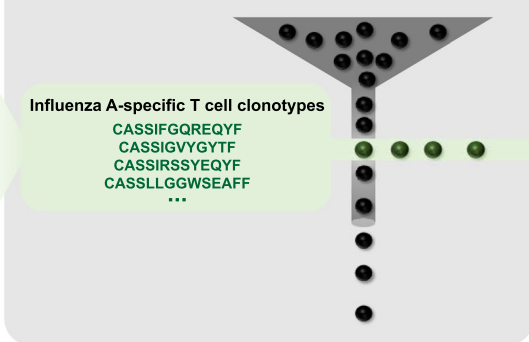


© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

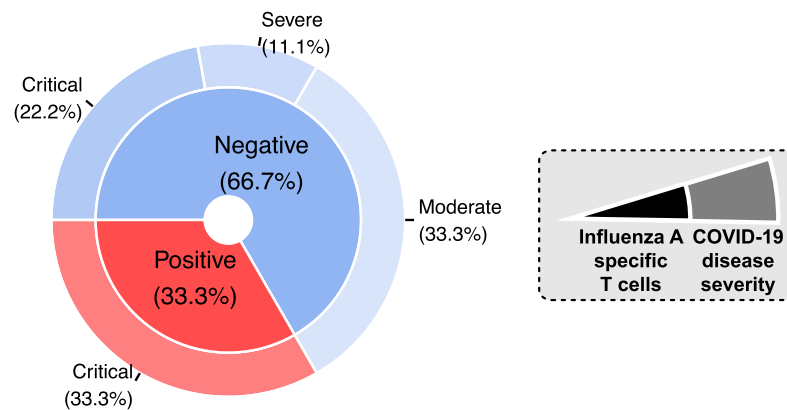
A Analysis framework

Chen, G., et al. (2017). *Cell Rep* 19, 569-583.
 Dash, P., et al. (2017). *Nature* 547, 89-93.
 Gil, A., et al. (2015). *J Virol* 89, 4102-4116.
 Glanville, J., et al. (2017). *Nature* 547, 94-98.
 Grant, E.J., et al. (2016). *J Biol Chem* 291, 24335-24351.
 Sant, S., et al. (2018). *Front Immunol* 9, 1453.
 Song, I., et al. (2017). *Nat Struct Mol Biol* 24, 395-406.
 van de Sandt, C.E., et al. (2019). *Nat Commun* 10, 5579.
 Wang, Z., et al. (2018). *Nat Commun* 9, 824.
 Watkin, L.B., et al. (2017). *J Allergy Clin Immunol* 140, 1206-1210.
 10X genomics, 2019.

Digitalized sorting of epitope-specific T cells



B Bronchoalveolar T cells in COVID-19 patients



C Epitope-specific T cells in COVID-19 patients

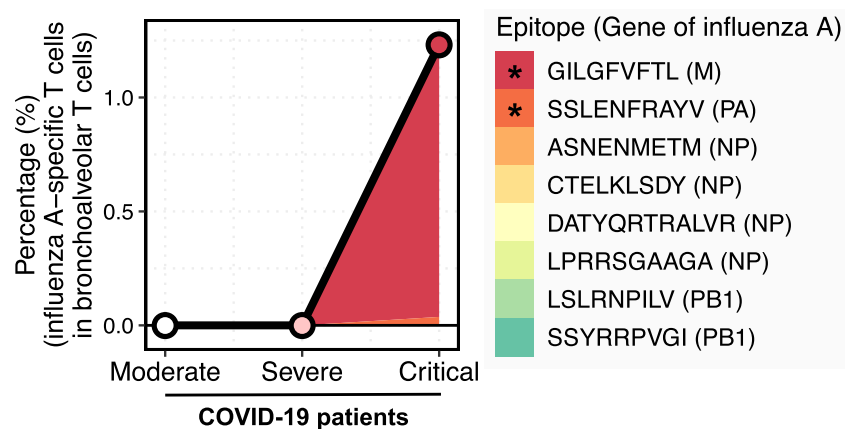


Fig. 1 Influenza A-specific T cell clonotypes in the bronchoalveolar lavage fluid T cells from COVID-19 patients. **A** The analysis framework of the digitalized sorting of epitope-specific T cells. Epitope-specific T cells were sorted according to their antigenic-specific TCR-β sequence. In this study, influenza A-specific T cell clonotypes were defined by T cell clonotypes with influenza A-specific TCR-β sequence using single-cell RNA sequencing. **B** The donut plot shows the percentage of COVID-19 patients defined by the categories of influenza A-specific T cells and COVID-19 disease severity. The colors represent the COVID-19 patients with or without influenza A-specific T cells in bronchoalveolar lavage fluid T cells. **C** The percentage (%) of influenza A-specific T cells in bronchoalveolar T cells in COVID-19 patients with mild (n = 3), severe (n = 1), and critical (n = 5) disease severity, with color coding according to the specificity of T cells to different epitopes from different genes of influenza A virus. The "*" shows the epitopes that could be found to elicit T cell response

of eight influenza A-epitopes, GILGFVFTL and SSLEN-FRAYV, could elicit T cell response in critically severe COVID-19 patients.

The co-infection of COVID-19 with influenza might complicate the diagnosis, treatment, and prognosis of COVID-19, posing potential challenges to public health. In this study, we provide the first evidence in human bronchoalveolar microenvironment that anti-influenza T cells exist in critically severe COVID-19 patients, but not patients with moderate disease severity. The existence of anti-influenza T cells specifically indicates the immunological status for influenza infection. This finding reveals that the co-infection of SARS-CoV-2 with influenza might cause more severe illness, suggesting that the prevention of influenza infection during the current SARS-CoV-2 pandemic might be important for reducing casualties caused by COVID-19. Considering the upcoming flu season will inevitably merge with the current COVID-19 pandemic, influenza vaccination might be beneficial in high-risk populations of COVID-19 infection. Additionally, the surveillance of influenza infection should be considered in COVID-19 patients.

Acknowledgements

We thank Dr. Zhang and his colleagues for the data of single-cell T cell receptor sequencing from their previously published study that were deposited in Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) under the accession number GSE145926.

Authors' contributions

M.Z. conceived the project, developed the method, conducted data analysis, and wrote the manuscript. The author read and approved the final manuscript.

Funding

This project was supported by the National Natural Science Foundation of China (32100739) to Dr. Ming Zheng.

Availability of data and materials

The data that support the findings of this study will be available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate

No human subjects were directly involved in this study. All the data used in this study were derived from existing de-identified biological samples from prior studies. Thus, ethical and patient consent was not required in this study.

Consent for publication

Not applicable.

Competing interests

This study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author details

¹Institute of Military Cognition and Brain Sciences, Academy of Military Medical Sciences, 27 Taiping Road, Beijing 100850, China. ²Beijing Institute of Basic Medical Sciences, 27 Taiping Road, Beijing 100850, China.

Received: 4 November 2021 Accepted: 16 December 2021
Published online: 20 December 2021

References

1. Dadashi M, Khaleghnejad S, Abedi Elkhichi P, Goudarzi M, Goudarzi H, Taghavi A, Vaezjalali M, Hajikhani B. COVID-19 and influenza co-infection: a systematic review and meta-analysis. *Front Med (Lausanne)*. 2021;8:681469.
2. Zheng M. ACE2 and COVID-19 susceptibility and severity. *Aging Dis*. 2022.
3. Kinoshita T, Watanabe K, Sakurai Y, Nishi K, Yoshikawa R, Yasuda J. Co-infection of SARS-CoV-2 and influenza virus causes more severe and prolonged pneumonia in hamsters. *Sci Rep*. 2021;11(1):21259.
4. Zheng M, Zhang X, Zhou Y, Tang J, Han Q, Zhang Y, Ni Q, Chen G, Jia Q, Yu H, et al. TCR repertoire and CDR3 motif analyses depict the role of alpha-beta T cells in Ankylosing spondylitis. *EBioMedicine*. 2019;47:414–26.
5. Dalai SC, Baldo L. Letter to the editor regarding 'perspective: diagnostic laboratories should urgently develop T cell assays for SARS-CoV-2 infection.' *Expert Rev Clin Immunol*. 2021;17:1155–7.
6. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med*. 2020;26(6):842–4.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

