Single-cell RNA sequencing-based immunological feature analysis of a COVID-19 patient with recurrent positive SARS-CoV-2 RNA

Bin Su[1], Ruya Sun[2], Lili Zhang[1], Jiaying Zhang[1], Xia Feng[1], Zhenhuan Cao[1], Wei Hou[1], Xianghua Guo[1], Qing Ge[3], Qinghua Cui[2], Yuan Zhou[2], Yingmin Ma[1], Ronghua Jin[1,4], Yingmei Feng[1]

[1]Beijing Youan Hospital, Capital Medical University, Beijing 100069, China;
[2]Department of Biomedical Informatics, School of Basic Medical Sciences, Key Laboratory of Molecular Cardiovascular Sciences of the Ministry of Education, Center for Non-coding RNA Medicine, Peking University Health Science Center, Beijing 100191, China;
[3]Department of Immunology, Key Laboratory of Medical Immunology, Ministry of Health, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China;
[4]Beijing Ditan Hospital, Capital Medical University, Beijing 100102, China.

To the Editor: Since the outbreak in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected >2.4 billion people worldwide, resulting in >4.9 million deaths. To understand the pathogenesis of SARS-CoV-2 infection, single-cell RNA-sequencing (scRNA-seq) profiles of peripheral blood mononuclear cells (PBMCs) have been constructed in coronavirus disease-2019 (COVID-19) patients at different disease stages.[1] These data showed typical interferon response and expansion of cytotoxic effector T cell subsets in moderate patients.[1] After progression to severe type, plasmablasts and a noncanonical neutrophil subset were elevated in patients with acute respiratory distress syndrome.[1] Conversely, patients in the recovery stage had increased plasma cells with novel B cell-receptor changes.[2]

Intriguingly, a meta-analysis of 5182 convalescent patients showed that the cumulative rate of recurrent RNA positivity was 12% (95% CI: 12%–13%) after 1 month and 2 months of discharge.[3] To uncover the underlining immunological features, we explored the transcriptomic immune landscape by scRNA-seq using PBMCs from a convalescent patient with recurrent positive RNA extracted from pharyngeal swabs. Using a quantitative polymerase chain reaction assay, the positive SARS-CoV-2 RNA was identified after 14 days of discharge and lasted for another 8 weeks in the patient. Convalescent COVID-19 patients ($n = 15$) with negative SARS-CoV-2 RNA during follow-up and patients who died from COVID-19 ($n = 4$) were included as controls.

The general characteristics of the study patients are presented in Supplementary Table 1, http://links.lww.com/CM9/A901. Of 20 COVID-19 patients analyzed, the average age was 65.1 years (range: 33–92 years) and eight were females. When classified by disease severity, the number of moderate, severe, or critically severe was amounted to 3, 12, or 5, respectively. Considering that one of three patients with a moderate type of COVID-19 had recurrent SARS-CoV-2 RNA positivity, the B cell receptor (BCR) in single cells of these patients was sequenced using 10× platform. T cell receptor (TCR) of all enrolled patients was also sequenced, in which the transcriptome of one sample was excluded from downstream analysis because of the "low fraction reads in cells" alert displayedinthe Cell Ranger websummary. A flowchart is presented in Figure 1A.

No virus read was mapped in PBMC samples. After sequence processing and quality control [Supplementary Figure 1, http://links.lww.com/CM9/A901], an immune atlas was generated, which comprised 267,901 high-quality cell transcriptomes with an average of 14,100 PBMCs per sample. Normalization and clustering were subsequently run-on cell-by-genes expression matrix. For visualization, uniform manifold approximation and projection dimensional reduction were run on identified clusters. Based on the expression level of canonical cell markers and highly expressed differential expressed genes (DEGs) of each cluster, eight major cell types were identified: T cells (CD3D, CD3E), B cells (CD79A), natural killer (NK) cells (NKG7, GNLY, FCGR3A), monocytes (CD14, FCGR3A), monocyte-derived dendritic cells (MO-DCs; CD1C), plasmacytoid dendritic cells

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001956

Bin Su and Ruya Sun contributed equally to the work.
Correspondence to: Dr. Yingmei Feng, Department of Science and Technology, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China
E-Mail: yingmeif13@sina.com

Chinese Medical Journal 2022;135(8)
Received: 26-10-2021; **Online:** 15-02-2022 **Edited by:** Yuanyuan Ji

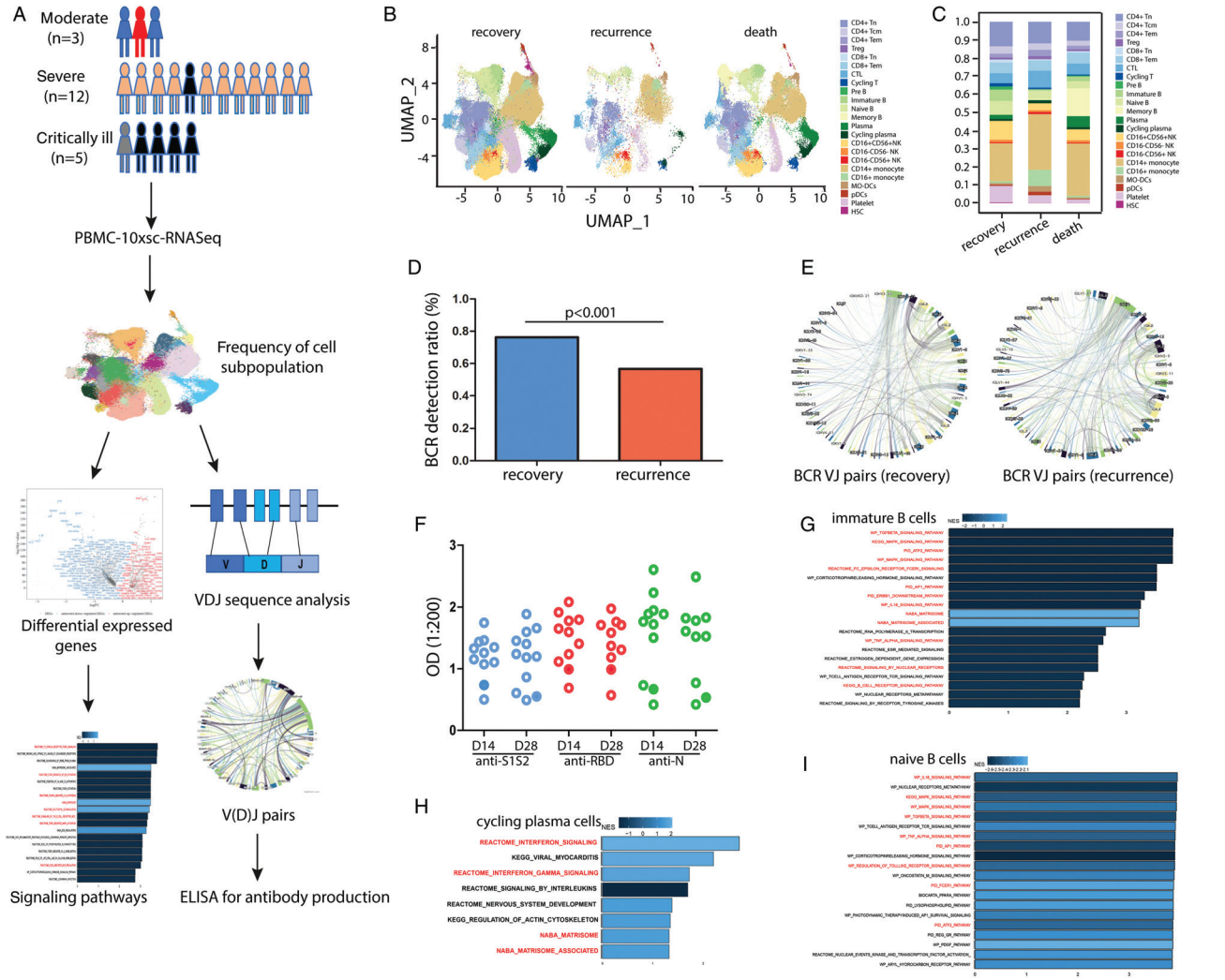

Figure 1: Immunological features of a COVID-19 patient with recurrent positive SARS-CoV-2 RNA. (A) Flowchart. (B) Uniform manifold approximation and projection plot of labeled 23 cell subpopulations among 267,901 single cells collected from PBMC of recovery ($n = 15$), recurrence ($n = 1$), and death ($n = 4$) COVID-19 patient. (C) Bar plot showed a relative distribution of cell subpopulations by different outcomes. (D) BCR detection ratio in recovered and recurrent patients. Pearson's χ^2 test and Yates' continuity correlation were used for analysis. (E) Sankey wheel plot showing V-J pair distribution in BCR repertoire of COVID-19 moderate infection patients with recovery (left) or recurrent (right) outcomes. Each line represents the Vgene-J gene pair of a single cell. (F) Plasma levels of antibody against SARS-CoV-2 with a dilution of 1/200. Filled dot corresponded to the result of recurrent patient. (G-I) Bar plots showing top enriched GSEA terms for DEGs of immature B cells (G), cycling plasmas (H), and naïve B cells (I) and in the recurrent patient. Value of X axis represented -log10 (*P* value). Bar color denoted the value of the normalized enrichment score. Interested pathways were indicated with red font. BCR: B cell receptor; COVID-19: Coronavirus disease-2019; DEGs: Differential expressed genes; GSEA: Gene set enrichment analysis; PBMC: Peripheral blood mononuclear cell; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

(pDCs; IL3RA, GZMB), hemopoietic stem cells (CYTL1), and platelets (PPBP, PF4, GP9). Pearson's χ^2 test showed that the proportions of B cells and NK cells were decreased but the proportions of T cell, monocyte, MO-DCs, and pDCs in PBMC proportion were increased in the recurrent patient compared with those recovered or died from COVID-19 (corrected $P < 0.05$).

To dissect cell subpopulations, the unbiased cell type recognition tool SingleR package was applied to confirm manual cell annotation. Cell components with a frequency >5% in all outcome groups (ie, T cells, B cells, NK cells, and monocytes) were re-clustered and annotated as corresponding subtypes following a similar pipeline. B cells were categorized by classical markers into pre-B cells (IL7R+), immature B cells (CD37+, MS4A1+), naïve B cells (MS4A1+, IGHD+), memory B cells (CD27+, BLNK+), plasma cells (MZB1+, IGHG1+, TNFRSF17+), and cycling plasma cells (MZB1+, IGHG1+, TNFRSF17+, MKI67+), respectively [Figure 1B]. By scRNA-seq analysis, the frequency of pre-B cells, immature B cells, naïve B cells, and plasma cells was 30.0-, 8.7-, 1.7- and 9.2-fold lower in the recurrent patient, respectively, compared with the recovered ones. The frequencies of memory B cells and cycling plasma cells among PBMC were significantly higher in the recurrent patient compared with the recovered subjects [Figure 1C].

To explore BCR clonal composition, BCR V(D)J sequencing analysis (10× Genomics) was processed using Cell Ranger and Seurat. A BCR repertoire map was constructed on three patients with moderate type. Of totalled 2570 B cells included in the above single-cell transcriptome analysis, BCR sequence and V(D)J pair gene profile were obtained in 2192 cells (85.29%). Among 2192 B cells, 1751 cells (68.13%) that had both productive heavy

chain (IGH) and light chain (IGL, IGK) of BCRs without dual express of TCR and BCR were screened out for downstream analysis.

Among B cell subpopulations, the averaged BCR detection ratio ranged from 0 for pre-B cells to 79.86% for naïve B cells within B cell subpopulations. However, the BCR detection ratio was 56.7% for the recurrent patient and 76.4% for the recovered subjects ($P < 0.05$) [Figure 1D]. By comparing the clone status, the overall BCR repertoire of recovered patients presented an inconspicuous clone expansion status. Nevertheless, only one cell had two clones among 610 clonotypes of the recurrent patient. Complementarity determining regions (CDRs) are variable regions of BCR and TCR, which bind to the epitopes of antigens. Among CDRs, CDR3 is the most variable region.[4] Our data revealed that the CDR3 sequence was shared in the top four IGL and IGK pools, but not in the top three IGH pools across three outcome groups. However, a length bias of CDR3 was presented in IGH ($P < 0.0001$) in the recurrent patient, which was not the case in the other groups. Furthermore, the most detected VJ pair in the recurrent patient was IGKV1-5 ~ IGKJ1, different from IGKV3-20 ~ IGKJ2 detected in the recovered group [Figure 1E]. Accordingly, plasma levels of anti-S1S2, anti-RBD, and anti-N proteins were lower in the recurrent patient in comparison with the others [Figure 1F]. Put together, all the data suggested impaired B cell development and function in the recurrent patient.

To further elucidate the potential mechanisms associated with impaired B cell development and function in the recurrent patient, gene set enrichment analysis (GSEA) was subsequently performed based on significant DEGs (adjusted $P < 0.05$). GSEA results illustrated that Toll-like receptor 4 cascade signaling, which is required for B cell activation,[5] was decreased in immature B cells, naïve B cells, and cycling plasma cells of the recurrent patient ($P < 0.05$, adjusted $P < 0.25$, NES < 0). NF-kB plays a mandatory role in promoting immature B cell survival and mature B maturation, but it was downregulated downstream of the reduced BCR and Fc-ε receptor I signaling pathways in the recurrent patient [Figure 1G-I].

In summary, our data demonstrated impaired B cell development, proliferation, and function in the recurrent patient, all of which were attributable to weakened antigen recognition. From the mechanistic viewpoint, the reduced TNF/TNF receptors pathways, NF-kB activation, and PLAUR/integrin $\alpha_M\beta_2$ could contribute to deficient B cell development, proliferation, and migration in the recurrent patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Funding

This work was supported by grants from the Beijing Science Foundation of China (No. Z201100005520062, No. Z201100007920017), the National Natural Science Foundation of China (No. 81772165), and the China Primary Health Care Foundation-Youan Medical Development Fund (No. BJYAYY-2020PY-01).

Conflicts of interest

None.

References

1. Zhang JY, Wang XM, Xing X, Xu Z, Zhang C, Song JW, *et al*. Single-cell landscape of immunological responses in patients with COVID-19. Nat Immunol 2020;21:1107–1118. doi: 10.1038/s41590-020-0762-x.
2. Wen W, Su W, Tang H, Le W, Zhang X, Zheng Y, *et al*. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov 2020;6:31. doi: 10.1038/s41421-020-0168-9.
3. Mattiuzzi C, Henry BM, Sanchis-Gomar F, Lippi G. SARS-CoV-2 recurrent RNA positivity after recovering from coronavirus disease 2019 (COVID-19): a meta-analysis. Acta Biomed 2020;91(3): e2020014. doi: 10.23750/abm.v91i3.10303.
4. Xu JL, Davis MM. Diversity in the CDR3 region of V(H) is sufficient for most antibody specificities. Immunity 2000;13:37–45. doi: 10.1016/s1074-7613(00)00006-6.
5. Cheng S, Wang H, Zhou H. The role of TLR4 on B cell activation and anti-b(2)GPI antibody production in the antiphospholipid syndrome. J Immunol Res 2016;2016:1719720. doi: 10.1155/2016/1719720.

How to cite this article: Su B, Sun R, Zhang L, Zhang J, Feng X, Cao Z, Hou W, Guo X, Ge Q, Cui Q, Zhou Y, Ma Y, Jin R, Feng Y. Single-cell RNA sequencing-based immunological feature analysis of a COVID-19 patient with recurrent positive SARS-CoV-2 RNA. Chin Med J 2022;135:1000–1002. doi: 10.1097/CM9.0000000000001956