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Cellular and Molecular Atlas of Peripheral Blood Mononuclear Cells from a Pregnant Woman After Recovery from COVID-19

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Abstract

Objective: This study aimed to investigate the immune response of a pregnant woman who recovered from the coronavirus disease 2019 (COVID_RS) by using single-cell transcriptomic profiling of peripheral blood mononuclear cells (PBMCs) and to analyze the properties of different immune cell subsets.

Methods: PBMCs were collected from the COVID_RS patient at 28 weeks of gestation, before a cesarean section. The PBMCs were then analyzed using single-cell RNA sequencing. The transcriptional profiles of myeloid, T, and natural killer (NK) cell subsets were systematically analyzed and compared with those of healthy pregnant controls from a published single-cell RNA sequencing data set.

Results: We identified major cell types such as T cells, B cells, NK cells, and myeloid cells in the PBMCs of our COVID_RS patient. The increase of myeloid and B cells and decrease of T cells and NK cells in the PBMCs in this patient were quite distinct compared with that in the control subjects. After reclustering and Augur analysis, we found that CD16 monocytes and mucosal-associated invariant T (MAIT) cells were mostly affected within different myeloid, T, and NK cell subtypes in our COVID_RS patient. The proportion of CD16 monocytes in the total myeloid population was increased, and the frequency of MAIT cells in the total T and NK cells was significantly decreased in the COVID-RS patient. We also observed significant enrichment of gene sets related to antigen processing and presentation, T-cell activation, T-cell differentiation, and tumor necrosis factor superfamily cytokine production in CD16 monocytes, and enrichment of gene sets related to antigen processing and presentation, response to type II interferon, and response to virus in MAIT cells.

Conclusion: Our study provides a single-cell resolution atlas of the immune gene expression patterns in PBMCs from a COVID_RS patient. Our findings suggest that CD16-positive monocytes and MAIT cells likely play crucial roles in the maternal immune response against severe acute respiratory syndrome coronavirus 2 infection. These results contribute to a better understanding of the maternal immune response to severe acute respiratory syndrome coronavirus 2 infection and may have implications for the development of effective treatments and preventive strategies for the coronavirus disease 2019 in pregnant women.

Keywords: Transcriptome; Single-cell analysis; Pregnancy; Peripheral blood mononuclear cells; Recovery; COVID-19

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a major impact on human health. During pregnancy, COVID-19 can present with a spectrum of illnesses, ranging from experiencing no symptoms to developing severe/critical disease.[1–3] Pregnant patients with COVID-19 are more likely to experience obstetric complications such as preeclampsia and preterm birth.[4–6]

Infection with SARS-CoV-2 can activate the antiviral host immune response, resulting in different disease severity and outcomes. Distinguishing features of severe SARS-CoV-2 infection include severe inflammatory responses and impaired innate and adaptive immune responses.[7,8] Furthermore, single-cell RNA sequencing (scRNA-seq) analyses have identified transcriptomic properties of immunological responses in COVID-19 based on the Blood Atlas.[9–11] A deeper understanding of the disease's immunopathology has been gained from these studies.

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Pregnancy is a unique immunological state accompanied by a balance of maternal immune tolerance and suppression to tolerate the semiallogeneic fetus,[12,13] and viral infection poses a major challenge by threatening to alter this immune balance.[14] Recent work on the transcriptome of cells at the maternal-fetal interface in humans has shed new light on the fundamental signatures of the placental defense mechanisms for SARS-CoV-2 infection.[15–17] Notably, several studies have further characterized changes in immune responses and conducted phenotypic analysis of leucocytes against SARS-CoV-2 infection in the maternal peripheral blood using high-throughput proteomics liquid chromatograph-mass spectrometer–based multiomics analysis, multiplex immunoassays, or fluorescence activating cell sorter for profiling of proteins, lipids, and metabolites.[16,18,19] However, a comprehensive characterization of the immune response of peripheral blood mononuclear cells (PBMCs) to SARS-CoV-2 infection in pregnancy has not been well described at the single-cell transcriptomic level.

The present study examined PBMCs from a pregnant woman who recovered from COVID-19 (COVID_RS) using scRNA-seq and systematically compared the immunological characteristics with healthy pregnancy from published data sets, providing new clues or a better understanding of the blood immune defense response against SARS-CoV-2 during pregnancy.

Materials and methods

Data collection and processing in scRNA-seq

The study was approved by the medical ethics committee of The Third Affiliated Hospital of Guangzhou Medical University, Medical Research (no. 2020090) and the participant signed informed consent.

The whole process of infection, treatment, and sample collection of a pregnant COVID-19 case have been well described in our previous study.[15] Briefly, the patient (COVID_RS) was initially asymptomatic for SARS-CoV-2 at 20 weeks of gestation and then experienced mild illness, followed by severe illness until the recovery stage, until finally the pregnancy had to be terminated because of an incomplete uterine rupture at gestational week 28. PBMCs were extracted from whole blood using density gradient centrifugation before a cesarean section and prepared for scRNA-seq using the Chromium Single Cell 5′ Reagent version 2 kit (10× Genomics, Pleasanton, CA, USA). After cDNA amplification, libraries were constructed using the Chromium Single Cell 5′ Reagent kit (10× Genomics) and Gel Bead Kit based

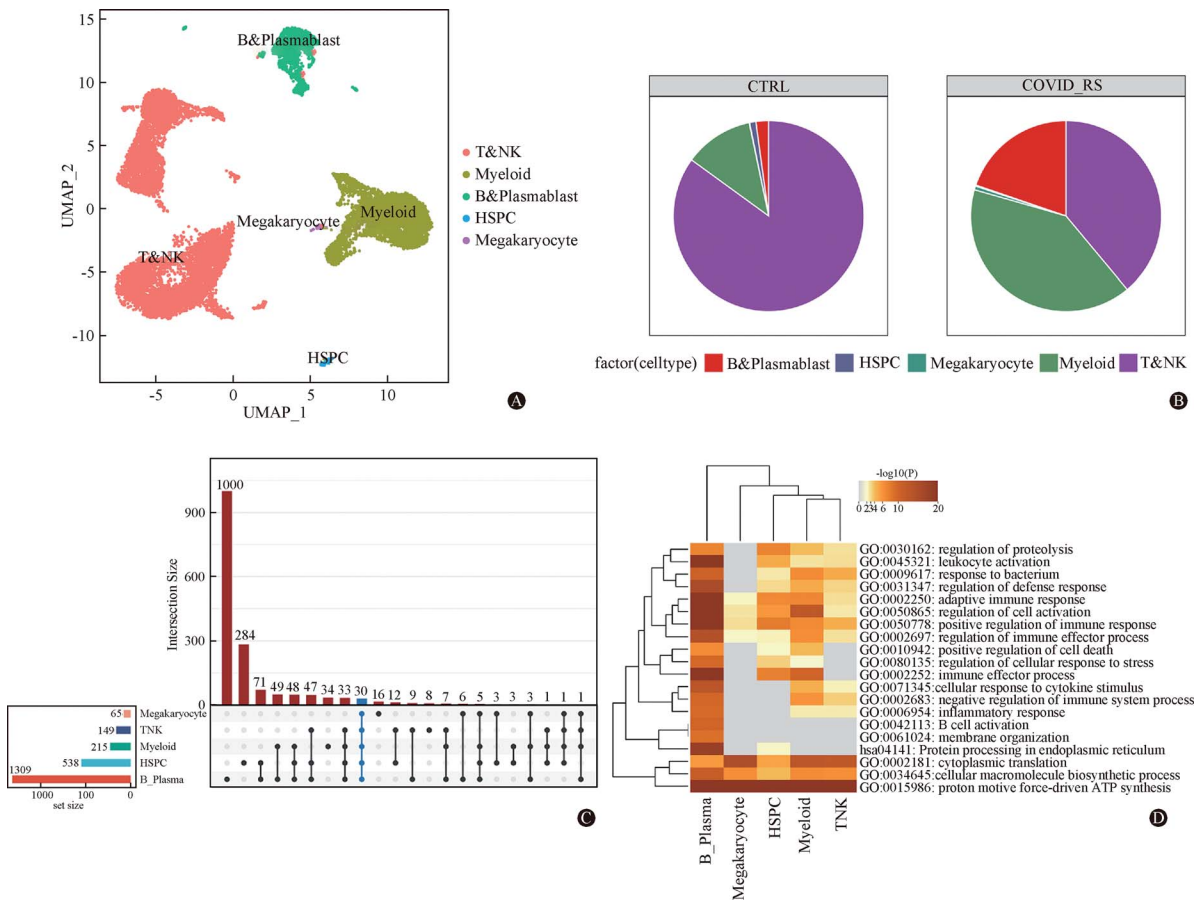

Figure 1. Single-cell transcriptomes profiling of PBMCs from a pregnant COVID_RS patient. A On the basis of the marker genes, five major cell types including T & NK, myeloid, B & plasmablast, HSPC, and megakaryocyte were identified using the UMAP plot. B Pie chart showing the proportion of each cell type in the COVID_RS case and control group. C UpSet plot of DEGs ($P < 0.01$ and absolute log-fold change > 2) between COVID_RS and CTRL among all cell types identified by scCODE. D Functional enrichment of DEGs among all cell types using Metascape. COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; DEG: Differentially expressed gene; HSPC: Hematopoietic stem and progenitor cell; NK: Natural killer; PBMCs: Peripheral blood mononuclear cells; UMAP: Uniform manifold approximation and projection.

on the manufacturer's protocol. Initial processing of the sequenced data was performed using CellRanger software.

Preprocessed scRNA-seq data of PBMCs from Vento-Tormo's scRNA-seq study[20] on five pregnant women (control group) were downloaded from OmicsDI Databases (Accession: E-MTAB-6701). The harmony function was used to combine two data sets and remove the batch effect,[21] followed by clustering cells based on scRNA-seq data using the Seurat V4 package.[22] In this study, clusters containing similar marker genes were initially merged into one cell type.

Myeloid, T, and NK cell reintegration

For myeloid, T, and NK cells, this study performed the second clustering to identify subsets of each cell type. In the second clustering, the same procedures were followed. Subsequently, the resulting cell types were annotated with cell subclusters based on known markers.

Identification of differentially expressed genes (DEGs) and function and pathway enrichment analysis

The scCODE package[23] (https://github.com/XZouProjects/scCODE) was used with the default settings to analyze the DEGs across different clusters between COVID_RS and controls. Through a variety of testing methods, scCODE can check the selected DEGs and improve the accuracy of single-cell DEGs analysis. DEGs with statistical significance were defined at $P < 0.01$ and absolute log-fold change (logFC) > 2 was applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis in Metascape[24] (http://metascape.org/gp/index.html#/main/step1).

Gene set variation analysis (GSVA)

The GSVA method was used with default settings to assign gene signature activity scores to individual cell type, as implemented in the GSVA v1.44.5 R package.[25]

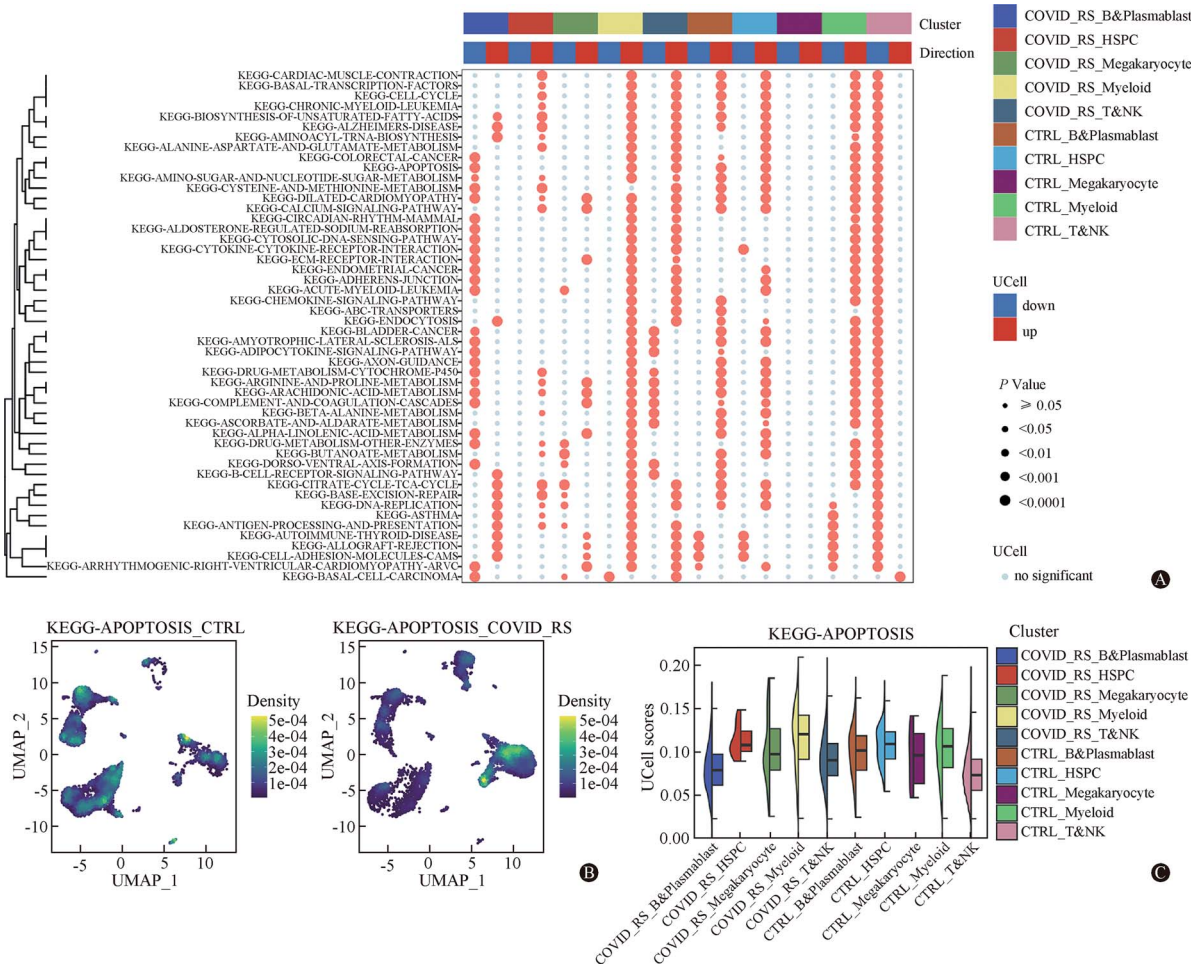

Figure 2. Identification of significantly related pathways by irGSEA. A Bubble plot showing co-upregulated or co-downregulated KEGG gene sets per cluster in the Ucell method by irGSEA analysis. B and C Density scatterplot and half vlnplot showing the expression and distribution of "KEGG-APOPTOSIS" in the Ucell method among all cell types in the COVID_RS case and control group. COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; KEGG: Kyoto Encyclopedia of Genes and Genomes; NK: Natural killer; HSPC: Hematopoietic stem and progenitor cells; UMAP: Uniform manifold approximation and projection.

Augur analysis

Using Augur, a tool that prioritizes a population's response to experimental perturbations, the analysis of cell type prioritization was completed in a stepwise manner.[26]

Identification of significantly related pathways by irGSEA

Integration of all single-cell rank-based gene set enrichment analyses was performed with irGSEA (R package, https://github.com/chuiqin/irGSEA/). A multiple gene set enrichment score matrix was generated by multiple gene set enrichment methods and the differentially expressed gene sets of each cell subpopulation in the enrichment fraction matrix of each gene set were calculated by the Wilcoxon test.

Results

Single-cell transcriptome profiling of PBMCs from a COVID_RS pregnant woman

To characterize the transcriptional profiling of PBMC cells in pregnant patients' recovery from COVID-19, we compared the scRNA-seq data sets of PBMC cells in a pregnant woman recovering from COVID-19 to the published scRNA-seq data sets of PBMCs from five pregnant women (control group). After quality control, a total of 16,894 cells were included for analysis, including 6968 cells from the COVID_RS case and 9926 cells from controls. After using the harmony function to integrate the two data sets and remove batch effects, cell clustering based on scRNA-seq data was performed using the Seurat V4 package, and clusters containing similar marker genes were initially merged into one cell type.

Based on the marker genes, we identified five major cell types including T and NK cells, myeloids, B and plasmablast cells, megakaryocyte cells, and hematopoietic stem and progenitor cells (HSPCs) (Fig. 1A). These defined cell clusters expressed the corresponding cell markers (Fig. S1A–C, http://links.lww.com/MFM/A25). To evaluate the immune system states, we first compared the proportion of the main five cell types in the COVID_RS case with the normal controls. We observed an expansion of myeloid and B cells and a decrease in T and NK cells in the PBMCs from this COVID_RS case compared with the control group (Fig. 1B).

Then, we identified the DEGs between this COVID_RS case and control group in each cell type by using scCODE (Supplementary Table 1, http://links.lww.com/MFM/A30). Our results showed that blood immune cells exhibited heterogeneous transcriptional changes between COVID_RS and control group based on the number of DEGs (Fig. 1C). GO analysis of these DEGs revealed their involvement in the immune response, and the inflammatory response related pathway and positive regulation of cell death, highlighting a high degree of similarity among the T, NK, and myeloid cells (Fig. 1D).

To further explore the enriched signaling pathways in each cell subtype, we performed rank-based gene set enrichment analysis using irGSEA. We found that T, NK, and myeloid cells showed the most significant changes for KEGG

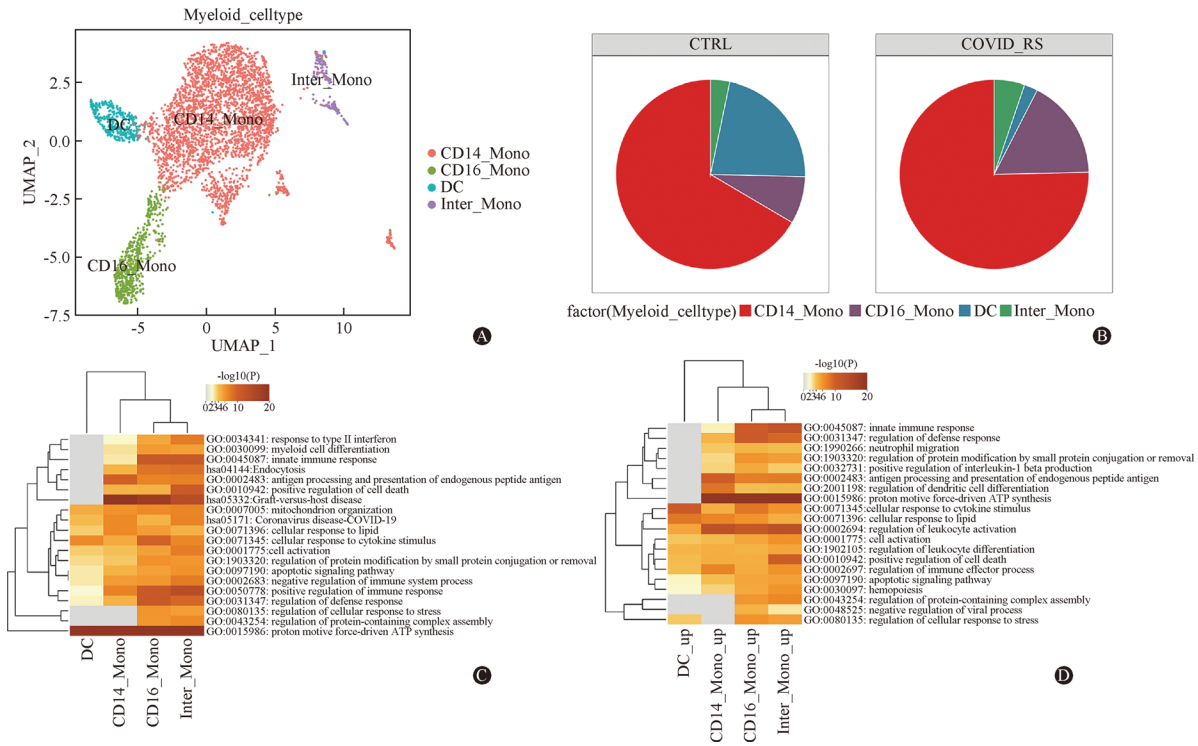

Figure 3. Immune profiles of myeloid cells in the COVID_RS patient. A UMAP visualization and clustering of four myeloid cell subtypes. B Pie chart showing the proportion of each myeloid cell subtype in the COVID_RS case and control group. C and D Functional enrichment of all (C) and upregulated DEGs (D) among the four myeloid cell subtypes using Metascape. DEG: Differentially expressed gene; COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; DC: CLEC9A+ CD1c+ dendritic cell; CD14_Mono: CD14high CD16– classical monocytes; CD16_Mono: CD14+/– CD16high nonclassical monocytes; Inter_Mono: CD14+ CD16+/– intermediate monocytes; UMAP: Uniform manifold approximation and projection.

pathway between COVID_RS and controls (Fig. S2A, http://links.lww.com/MFM/A26). Notably, more than half of all significantly enriched KEGG pathways in the T, NK, and myeloid cells were related to immunity, displaying an opposite trend between the two groups (Fig. S2B, http://links.lww.com/MFM/A26). Moreover, irGSEA analysis revealed the enrichment of cell cycle and apoptosis-related pathways in T, NK, and myeloid cells from COVID_RS case based on the Ucell method (Fig. 2A). In the COVID_RS case, the apoptosis pathway was upregulated in T, NK, and myeloid cells. By contrast, the apoptosis pathway was downregulated in T and NK cells but upregulated in myeloid cells in the control group (Figs. 2B, C), suggesting a cell type-specific response in COVID-19 recovery in pregnancy.

Immune profiles of myeloid cells in the COVID_RS patient

Myeloid cells are known to promote antigen presentation and inflammatory activities. To further characterize the myeloid subsets, we extracted and reclustered the data from myeloid cells. Four cellular subtypes were identified, namely CD14high CD16^{-} classical monocytes (CD14_Mono), CD14$^{+/-}$ CD16high nonclassical monocytes (CD16_Mono), CD14^{+} CD16$^{+/-}$ intermediate monocytes (Inter_Mono), and CLEC9A^{+} CD1c^{+} dendritic cell (DC) (Fig. 3A, Fig. S3A, http://links.lww.com/MFM/A27). Based on more precise classification of each subgroup, we next analyzed changes in the proportion of myeloid cells and found that DC were decreased, while CD14_Mono, CD16_Mono, and Inter_Mono cells were increased in the COVID_RS case (Fig. 3B). Additionally, using scCODE, we identified DEGs between the COVID_RS and control group in each myeloid subset (Supplementary Table 2, http://links.lww.com/MFM/A31). Few differential genes were common among these myeloid subsets between COVID_RS and controls (Fig. S3B, http://links.lww.com/MFM/A27).

The results of GO and KEGG pathway enrichment analysis of DEGs performed by Metascape showed that pathways related to antigen processing and presentation and regulation of cell death were enriched in all three monocyte cell types (Fig. 3C). Apoptotic signaling, cellular response to lipids, and cellular response to cytokine stimulus pathways were enriched in all four subtypes of myeloid cells, especially for the upregulated DEGs (Fig. 3D).

Furthermore, Augur analysis revealed that CD16 monocytes were mostly affected in the COVID_RS case (Fig. 4A).

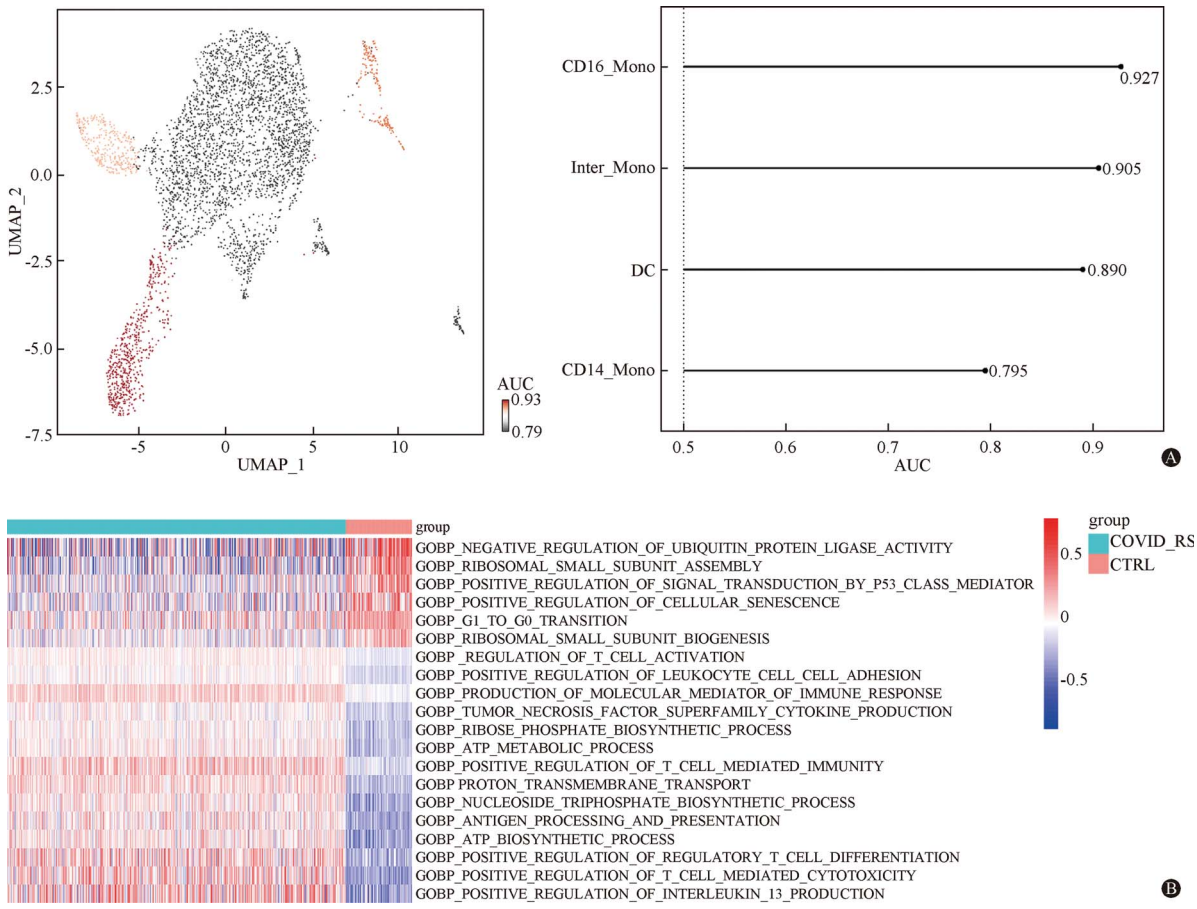

Figure 4. Functional characterization of CD16_mono subpopulation. A Augur analysis of cell type prioritization in the COVID_RS case among four myeloid cell subtypes. B The GSVA analysis of CD16_mono from the COVID_RS and control group. AUC: Area under the curve; CD14_Mono: CD14high CD16−classical monocytes; CD16_Mono: CD14+/− CD16high nonclassical monocytes; COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; DC: CLEC9A+ CD1c+ dendritic cell; GSVA: Gene set variation analysis; Inter_Mono: CD14+ CD16+/− intermediate monocytes; UMAP: Uniform manifold approximation and projection.

Therefore, we further analyzed the transcriptional signatures of CD16_mono between the COVID_RS and control groups and identified several upregulated DEGs enriched in positive regulation of immune response, antigen processing and presentation, regulation of type I interferon production, and regulation of defense response to virus pathways (Fig. S4A, http://links.lww.com/MFM/A28). Conversely, the downregulated DEGs were enriched in ATP synthesis, rRNA metabolic process, and negative regulation of tumor necrosis factor production (Fig. S4B, http://links.lww.com/MFM/A28). Furthermore, GSVA of the CD16_ mono subtype revealed a significant enrichment of genes related to antigen processing and presentation, T cell activation, T cell differentiation, T cell-mediated cytotoxicity, and tumor necrosis factor superfamily cytokine production, while genes related to cellular senescence and G1 to G0 transition were enriched in the control group (Fig. 4B). Moreover, irGSEA analysis showed that KEGG pathway including acute inflammatory response and activation of innate immune response was upregulated in the CD16_mono subtype in the COVID_RS case and downregulated in the control group (Figs. S4C, D, http://links.lww.com/MFM/A28).

Immune profiles of T and NK cells in the COVID_RS case

To further characterize the T and NK subsets, we extracted the data from T and NK cells. Eight cell subtypes were identified, including CD4$^+$ naive-CCR7$^+$, CD4 effector-GZMA$^+$ GZMB$^+$, CD8$^+$ naive-CCR7$^+$, CD8 effector-GZMA$^+$ GZMB$^+$ T cell subsets, mucosal-associated invariant T (MAIT)–SLC4A10$^+$ TRAV1–2, CD56briCD16$^-$ NK cells, CD56dimCD16$^+$ NK cells, and proliferative T/NK population-MKI67$^+$ (Fig. 5A, Fig. S5A, http://links.lww.com/MFM/A29). The composition of cell subsets across all cell lineages differed between the COVID_RS case and control group. Among the T and NK cells, CD4$^+$ naive and CD4$^+$ effector T cells were decreased, CD8$^+$ naive and CD8$^+$ effector T cells were increased, and the decrease was more prominent for the MAIT subset in the COVID_RS case. The COVID_RS case also had a greater proportion of the CD56bright NK population and proliferating T and NK cells populations than control group (Fig. 5B).

We identified DEGs between the COVID_RS case and control group in each T and NK subset using scCODE (Supplementary Table 3, http://links.lww.com/MFM/A32). The number of unique DEGs for each cell type and the number of common DEGs between or among the cell subtypes are shown in Figure 5C. Metascape analysis showed that these DEGs were enriched in antigen processing and presentation, allograft rejection, and ATP synthesis. In addition, regulation of apoptotic signaling pathways was also enriched in DEGs from MAIT and CD8 effector T and NK cell subsets (Fig. 5D).

Augur analysis showed that MAIT were the most affected among all T and NK subtypes in the COVID_RS case compared with the control group (Fig. 6A). To further explore the transcriptional changes in MAIT cells, we identified the upregulated and downregulated DEGs between the two groups using scCODE. We found that the upregulated DEG in COVID_RS case were significantly enriched in pathways related to oxidative phosphorylation, antigen processing and presentation, response to type II interferon, and response to virus. However, the downregulated DEGs

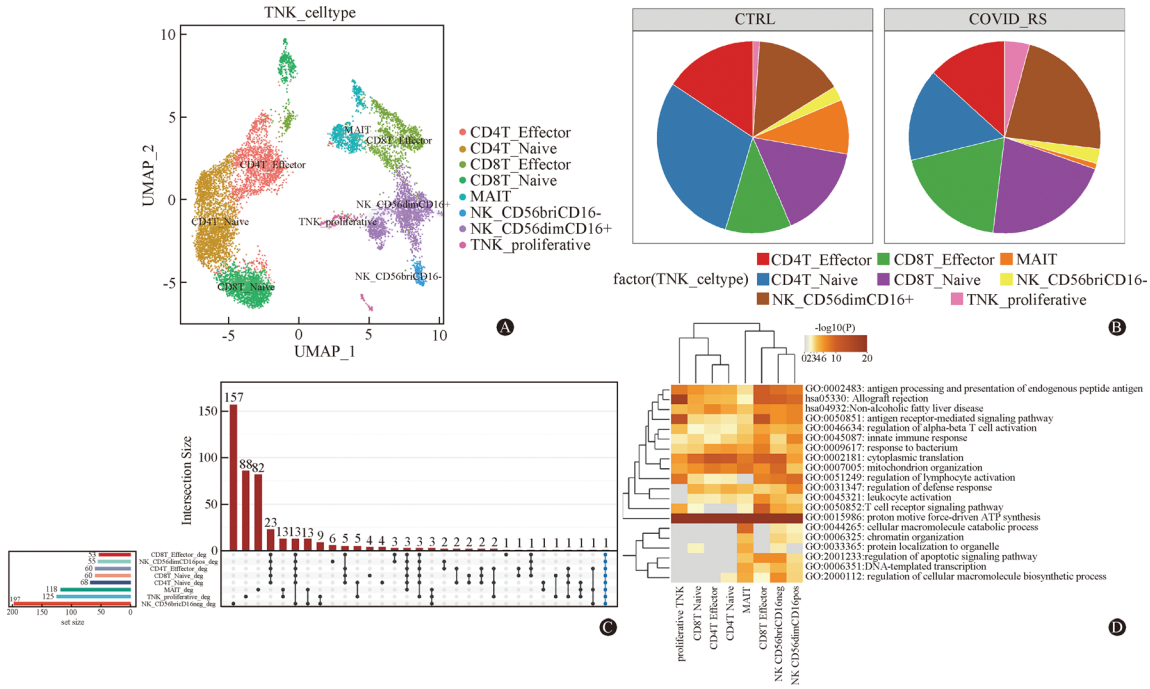

Figure 5. Immune profiles of T and NK cells in the COVID-RS patient. A UMAP visualization and clustering of eight T and NK subtypes. B Pie chart showing the proportion of each T and NK subtypes in the COVID_RS case and control group. C UpSet plot of DEGs ($P < 0.01$ and absolute log-fold change > 2) between the COVID_RS and control group among the eight T and NK subtypes identified by scCODE. D Functional enrichment of DEGs among the eight T and NK subtypes using Metascape. COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; DEG: Differentially expressed gene; MAIT: Mucosalassociated invariant T; NK: Natural killer; UMAP: Uniform manifold approximation and projection.

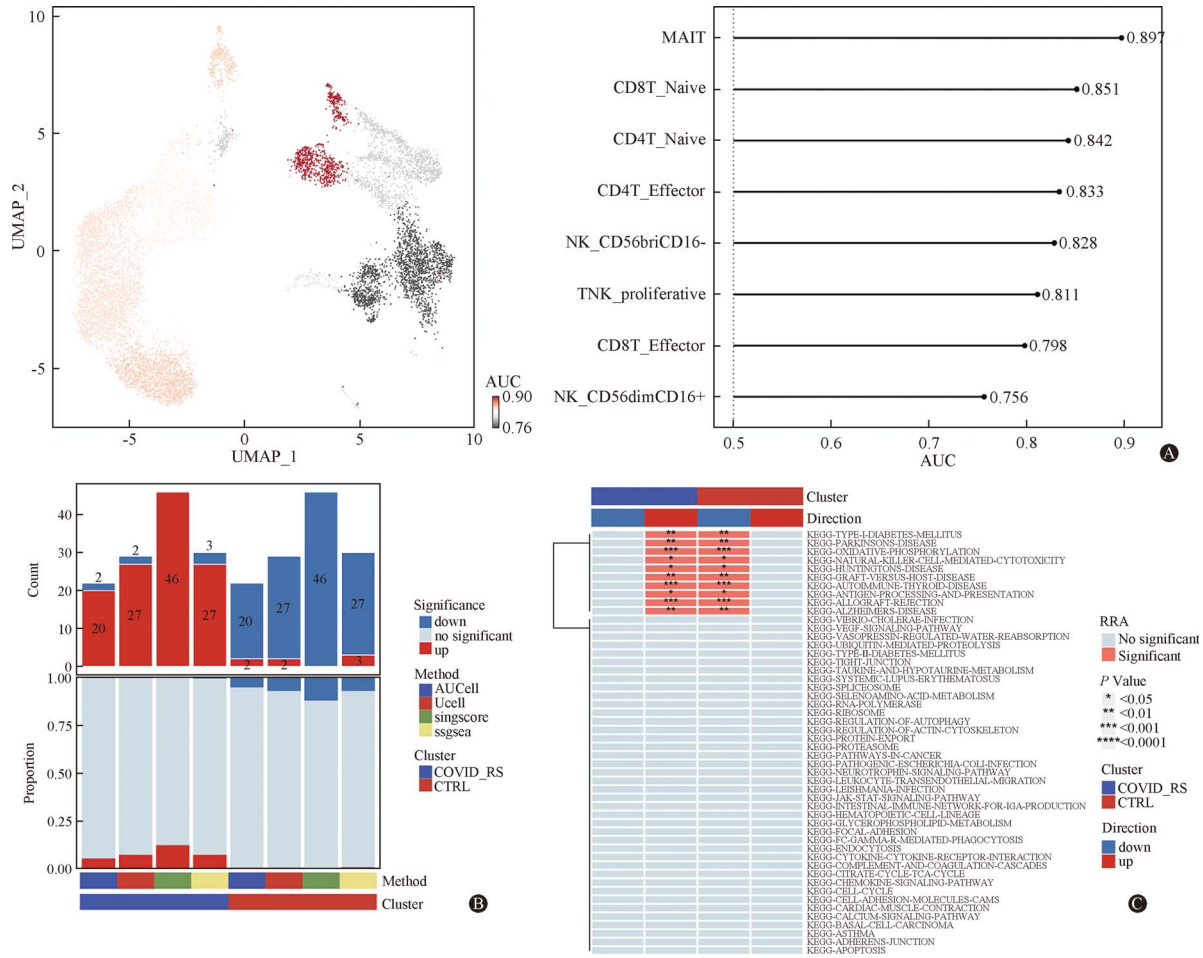

Figure 6. Functional characterization of T and NK cells. A Augur analysis of cell type prioritization in the COVID_RS case among the eight T and NK subtypes. B Stacked bar plot of the intersections of significant gene sets among the COVID_RS and control group in MAIT by all methods in the irGSEA analysis. C Heatmap plot of co-upregulated or co-downregulated KEGG gene sets in MAIT by the RRA method in irGSEA analysis. AUC: Area under the curve; COVID_RS: Pregnant woman recovered from the coronavirus disease 2019; CTRL: Control group; GSEA: Gene set enrichment analysis; KEGG: Kyoto Encyclopedia of Genes and Genomes; MAIT: Mucosal-associated invariant T; NK: Natural killer; RRA: Robust rank aggregation; UMAP: Uniform manifold approximation and projection.

were enriched in pathways related to ATP synthesis, DNA-templated transcription, mRNA metabolic process, and chromatin organization (Figs. S5B, C, http://links.lww.com/MFM/A29). We also performed irGSEA analysis to assess the enrichment of pathways in MAIT cells between the two groups. The analysis revealed that GO pathways related to T-cell proliferation, T-cell activation, and acute inflammation were upregulated in MAIT from the COVID_RS case, while they were downregulated in the control group (Fig. 6B, C).

Discussion

Although the single-cell transcriptome signature at the maternal-fetal interface and changes of inflammatory cytokines in the maternal peripheral blood against SARS-CoV-2 infection during pregnancy have been studied, a comprehensive characterization of the immune response of PBMCs to SARS-CoV-2 infection in pregnancy is still poorly understood. In this study, we performed scRNA-seq sequencing on PBMCs from a pregnant woman who recovered from COVID-19 and systematically compared the immunological characteristics with those of non-COVID-19 pregnancies from published data sets.[20]

Monocytes are the primary drivers of host immune defense against virus infection.[27] Several studies have proven the involvement of circulating monocytes in all stages of COVID-19.[28] In acute patients with severe SARS-CoV-2 infection symptoms, there is a significant reduction in the numbers of nonclassical (CD14dimCD16) and intermediate (CD14CD16) monocytes.[29,30] The increased ratio and level of classical CD14 IL-1β^{+} monocytes were found in PBMCs during early recovery of COVID-19.[31] During pregnancy, the proportion of monocytes slightly increases as gestational age advances,[32] reflecting the normal immune adaptation of pregnancy. However, we found that the total proportion of myeloid cells were increased in the COVID_RS case, with a more pronounced increase in the proportion of CD16^{+} monocytes. This suggests that the increase in monocyte proportion in the COVID_RS case is not solely because of the normal physiological changes during pregnancy,

but rather associated with the immune response to SARS-CoV-2 infection. Consistently, the pregnancy-specific increase in CD16 expression and elevated intermediate monocytes have been reported in response to SARS-CoV-2 infection, suggesting that a similar phenomenon is observed in women who have made a recovery from COVID-19.[33,34]

SARS-CoV-2 infection is known to cause lymphopenia, particularly affecting the systemic proportions of T cells.[35,36] It has been reported that pregnant women with SARS-CoV-2 infection also undergo a reduction in circulating T-cells, as evaluated by immunophenotyping methods.[16] We also found that the proportion of T cells in the COVID-RS case was reduced significantly compared with the control group, consistent with a previous study.[37] In addition, we found that pregnancy in the COVID-19 recovery phase had a unique phenotypic and functional profile of T cells. Specifically, the proportion of $CD4^+$ T and MAIT subsets were decreased, while the relative proportion of $CD8^+$ T cells was increased. Extensive research has shown excessive T-cell activation in patients with severe COVID-19,[7,35] which is consistent with COVID-RS–specific enrichment of T-cell activation–associated pathways, such as regulation of T-cell activation and T-cell receptor signaling pathway, observed in the T-cell subsets in the current study.

An interesting finding of this study is the significant decrease in the frequency of MAIT cells within the T and NK cell population in this COVID_RS case compared with healthy pregnant controls. The MAIT cells, recently defined as innate-like T cells, play an important role in the innate host defense against various bacterial and viral infections, as well as being activated by inflammatory stimuli without T-cell receptor–mediated antigen recognition.[38–40] Previous studies have indicated that pregnancy does not have a significant impact on the percentage of T-cell subsets in the peripheral blood.[32,41] However, under pathological conditions, the proportion of MAIT cells may be altered. A high proportion of MAIT cells was found in the PBMCs of patients who underwent recurrent miscarriages.[42] It is reported that the numbers of peripheral MAIT cells were persistently decreased and activated profoundly in patients with severe COVID-19 but recovered to normal levels during convalescence.[43,44] We observed a significant decrease in the proportion of MAIT cells among all T and NK cell subsets in the COVID_RS group, which is consistent with a previous report on pregnant patients that recovered from COVID-19 in late pregnancy.[37] Based on the transcriptomic profiling of MAIT cells in our study, enhanced apoptosis, decreased DNA transcription, and mRNA metabolism might have contributed to the decline of peripheral MAIT cells. Meanwhile, enrichment of pathways such as response to type II interferon and NK cell–mediated cytotoxicity showed activation of MAIT cells in the COVID_RS case. Collectively, these findings might provide hints to better understand the immunomodulatory phenotypes of T cell signatures in the maternal circulation to SARS-CoV-2 infection.

Taken together, our data have provided a single-cell resolution atlas of the gene expression patterns of the immune profiling of peripheral blood from a pregnant woman who recovered from COVID-19. The results revealed that CD16-positive monocytes and MAIT were the main affected immune cell subsets associated with COVID-19 and identified their functionally enriched gene signatures after recovery from SARS-CoV-2 infection.

Our study has some limitations. First, this was just a single case report. Therefore, the generalizability of the findings to other pregnant women with COVID-19 needs to be further investigated in studies with larger sample sizes. Second, the functional significance of DEGs identified in this study needs to be experimentally validated. Finally, it should be noted that the immune system undergoes dynamic changes during pregnancy, and these changes vary depending on the gestational age, which may impact the immune response to COVID-19. Despite these limitations, the study results provide hints to better understand maternal immune responses to SARS-CoV-2 infection. Subsequent studies should focus on different immune cell subset functions in COVID-19 pregnancy, including the CD16-positive monocyte and MAIT subsets.

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Author Contributions

Dunjin Chen and Jingsi Chen conceived and supervised the project and revised the manuscript. Lili Du performed data analysis and drafted the manuscript. Yingyu Liang participated in data analysis and drafting of the manuscript. Xiaoyi Wang, Lijun Huang and Xingfei Pan helped in sample collection and processing. All authors have read and approved the final manuscript.

Conflicts of Interest

None.

Editor Note

Dunjin Chen is the Associate Editor of *Maternal-Fetal Medicine*. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and the associated research groups.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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