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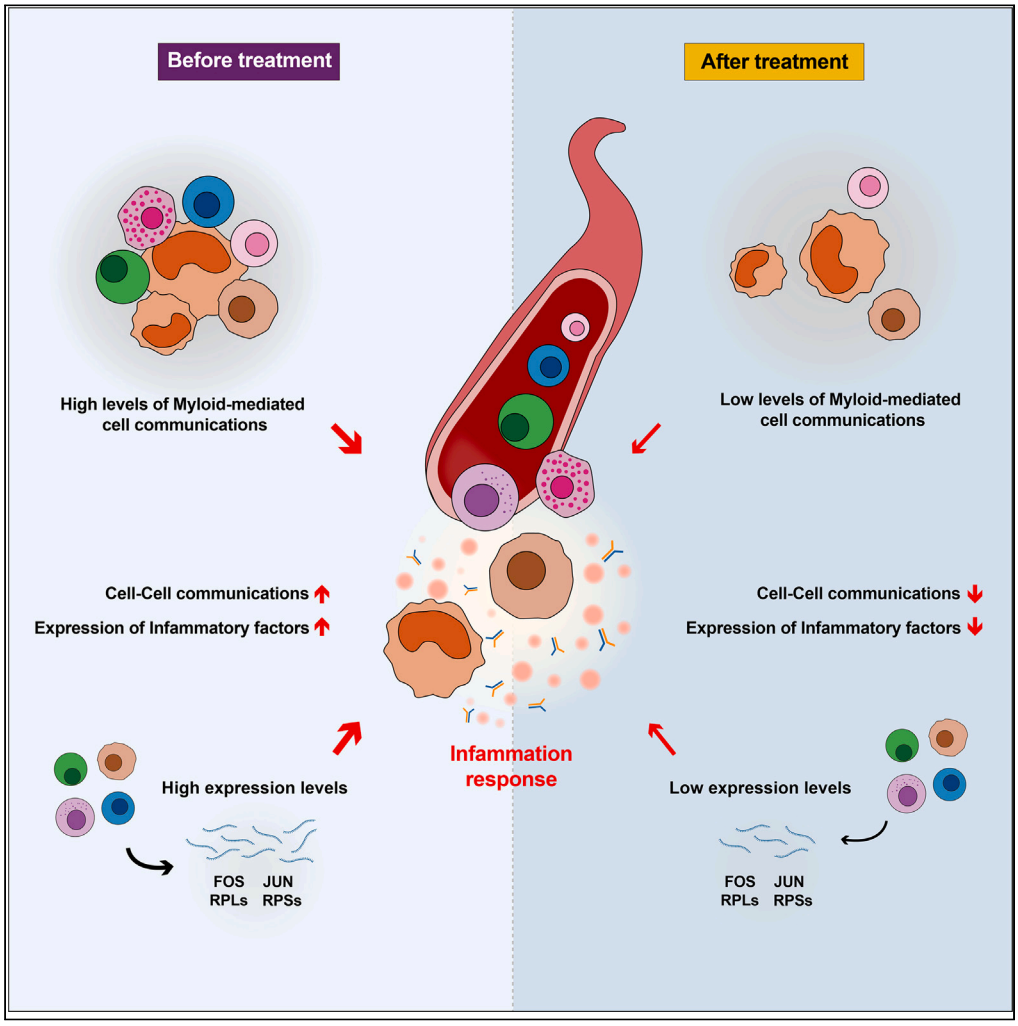

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Highlights

A single-cell immune atlas of patients with BD with quetiapine and valproate treatment

Decreased population of pro-inflammatory cells observed

Reduced ribosomal function detected in B and T cells after treatment

Myloid-mediated immune signaling pathway decreased after treatment

Qi et al., iScience 26, 107057
July 21, 2023 © 2023 The Authors.
https://doi.org/10.1016/j.isci.2023.107057

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Single-cell immune profiling reveals broad anti-inflammation response in bipolar disorder patients with quetiapine and valproate treatment

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SUMMARY

Bipolar disorder (BD) is a common mental disorder characterized by manic and depressive episodes. Mood disorders have been associated with immune dysfunction. The combination of quetiapine and valproate has shown positive effects in treating BD, but the impact on immune dynamics remains less understood. Using single-cell RNA sequencing, we observed that B cells exhibited downregulation of inflammation-related genes, while pro-inflammatory mast and eosinophil cells decreased following treatment. Ribosomal peptide production genes were found to be reduced in both B and T cells after treatment. Additionally, our findings suggest that the combined therapy effectively alleviates inflammation by reducing myloid-mediated immune signaling pathways. This study provides valuable insights into the immune atlas and uncovers a potential mechanism for immune disorder alleviation in patients with BD treated with quetiapine and valproate.

INTRODUCTION

Currently, bipolar disorder (BD) is a psychiatric illness affecting at least 1% of the world's population and is characterized by multiple-system disorder, including affective, emotional, cognitive, endocrine, autonomic nervous, and sleep functions.[1] Clinical deterioration of BD has been linked to alterations in immunology, including phase-dependent inflammatory response[2] and immune dysfunction.[3] Although clinical trials have have demonstrated the efficacy of combining quetiapine and valproate for improving bipolar depression in patients compared to other widely used drugs such as placebo and lithium and its potential impact on immune response,[4,5] few studies have investigated the specific effects of these drug treatments on changes in immunity, particularly immune cells.[6] Therefore, it is critical to study immunological changes during treatment to understand the mechanism of drugs and lay the foundation for developing potential therapeutic targets.

BD is a highly heterogeneous disease, but a significant number of patients experience neuro-progression, characterized by increasing frequency of mood episodes (manic or depressed) and a progressive decline in neurocognitive function.[7] Moreover, most patients with BD suffer from long-term chronic inflammation, which tends to be upregulated during acute mood periods.[8] Previous research studies have also found that the risk of autoimmune diseases is significantly higher in patients with BD.[9] Pro-inflammatory network mediated by monocyte tends to be enhanced in patients with BD, where both monocyte and T cell immune system are activated.[10] Activated immune cells can secrete inflammatory mediators such as cytokines and chemokines, which can stimulate neuron response to inflammation and lead to dysfunction.[11] Furthermore, immune cells component such as T and NK cells can also play a key role in progression of BD.[12] Mood stabilizers including valproate and second-generation antipsychotics such as quetiapine are currently main strategy to treat BD, as they have anti-inflammatory effects on patients and lead to signigicant improvement.[1,13,14] However, the deep mechanism of this treatment's anti-inflammatory effects is not well understood.

Single-cell RNA sequencing (scRNA-seq) now has become widely used to explore cellular and molecular dynamics in many fields and expand our understanding of the underlying mechanism of various diseases.

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https://doi.org/10.1016/j.isci.2023.107057

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Recent studies have successfully investigated the alterations of T cells in patients with BD using scRNA-seq technologies.[15] However, relevant data on BD research remain scarce, and knowledge of immune alterations in patients after applying quetiapine and valproate is still limited. To study immune dynamics during treatment in patients with BD, we employed scRNA-seq to profile a high-resolution immune landscape of patients after drug appalication. In the present study, we identified 10 major cell groups and analyzed the changes in B cells, T cells, NK cells, and monocyte separately, characterizing the cellular networks among these immune cells. Our results indicate that combined drug treatment can significantly reduce myloid-mediated inflammation response, which may alleviate inflammation-related emotional disorders. Overall, this study explored the immune changes in drug-treated patients, which could expand our understanding of the mechanisms of BD treatment and help identify effective therapeutic targets.

RESULTS

Overview of peripheral blood mononuclear cell (PBMC) immune atlas in patients with BD

A total of 55,572 cells were isolated and sequenced from PBMC suspensions obtained from eight samples, including four follow-ups before treatment (21,648 cells) and four follow-ups after treatment (33,924 cells) (Figure 1A). Cell types were mainly identified according to the expression of classical lineage markers of diverse clusters (Figure 1B). We identified 10 main cell types including myloid cells (CD14, LYZ), CD4 T cells (CD3D, CD4), CD8 T cells (CD3D, CD8A), proliferation T cells (CD3D, MKI67), NK cells (FCGR3A, KLRD1, GNLY), γδ T cell (CD3D, TRDC), B cells (CD79A, CD19), platelets (PPBP), hematopoietic stem cells (CD34), and RBC (HBB), which were clustered by uniform manifold approximation and projection(Figure 1C). We analyzed the distribution of immune cells between two groups and found that all cell types existed in both groups (Figure 1D). We then made statistics on the proportion of cell types and cell numbers of different samples and found no significant difference in proportion of cell types between two groups (Figures 1E and 1F).

Re-clustering and function analysis of B cells

To further understand the cell compartment of B cell, we re-clustered B cells and identified six distinct cell types, including switched memory B cell (CD19, CD27, IGHD-), naive B cell (CD19, IGHM, IGHD, $CD27^{-}$), memory B cell (CD27), plasmablast (CD19, CD27, TNFRSF17), CD14 atypical cell (CD14), and translational B cell (IGHM, IGHD, CD20) (Figures 2A and 2B). We calculated the cell proportion of B cell clusters between two groups and found that distinct B cell types showed no difference in proportion (Figure 2C). As previously reported, mitochondrial functions such as oxidative phosphorylation and ATP production contribute to B cell activation.[16] Once activated, antigens present, and cytokines secretion from B cells tend to increase largely, leading to a massive immune response and inflammation. To explore the difference in cell function after drug treatment, we analyzed the function of each cell type using gene set variation analysis. Our data suggested that genes involving in mitochondrial energy, ribosome peptide generation, and B cell activation are enriched in pre-treated group (Figure 2D, Table S3). Memory B cells and plasmablast play an important role in adaptive immunity, which may aggravate inflammatory reactions. Our results showed that inflammation-related genes, such as FOS and JUN, were expressed highly in memory B cells and plasmablast, respectively (Figure 2E). Genes involving in ribosomal protein synthesis, RPL13P12, RPS26, and RPL9P9, were expressed higher in pre-treated patients than those in treated patients (Figure 2E). Moreover, compared to those in post-treated patients, mitochondrial ATP synthesis genes, such as MTATP6P1 and MTCO1P12 and B cell activation-related genes, including IGHA1, IGHA2, and IGHG3, were primarily expressed in most B cells of the pre-treated group (Figure 2E). These observations suggest that quetiapine and valproate can reduce the protein synthesis, production of pro-inflammatory factors and inhibit the mitochondrial function of B cells, which may contribute to inflammation response.

Effect of treatment on T and NK cells

T and NK cells are critical in immune response, especially in cellular clearance and killing. Our re-clustering analysis identified 14 subsets of T and NK cells (Figure 3A) using previous identified markers (Figure 3B). NK cells could be divided into 3 subsets: cytotoxic NK (FCGR3A, NCAM1), NKT (CD3D), and regulatory NK (FCGR3A-, NCAM1). CD4 T cells could be divided into 4 clusters: CD4 central memory T cells (CCR7high), CD4 memory T cells (IL7Rhigh), CD4 naive T cells (CCR7high, CD27high), and Treg (IL2RA). CD8 T cell could be divided into 4 clusters: CD8 central memory T cells (CCR7), CD8 naive T cells (CCR7high, CD27high), CD8 effector memory T cells (GZMK), and CD8 effector T cells (CCR7-, IL7R-). Mucosal-associated invariant T cells were $KLRB1^{+}$, IL7Rhigh, and $GZMK^{+}$ cells. Although not statistically significant, cell proportion of CD4 central

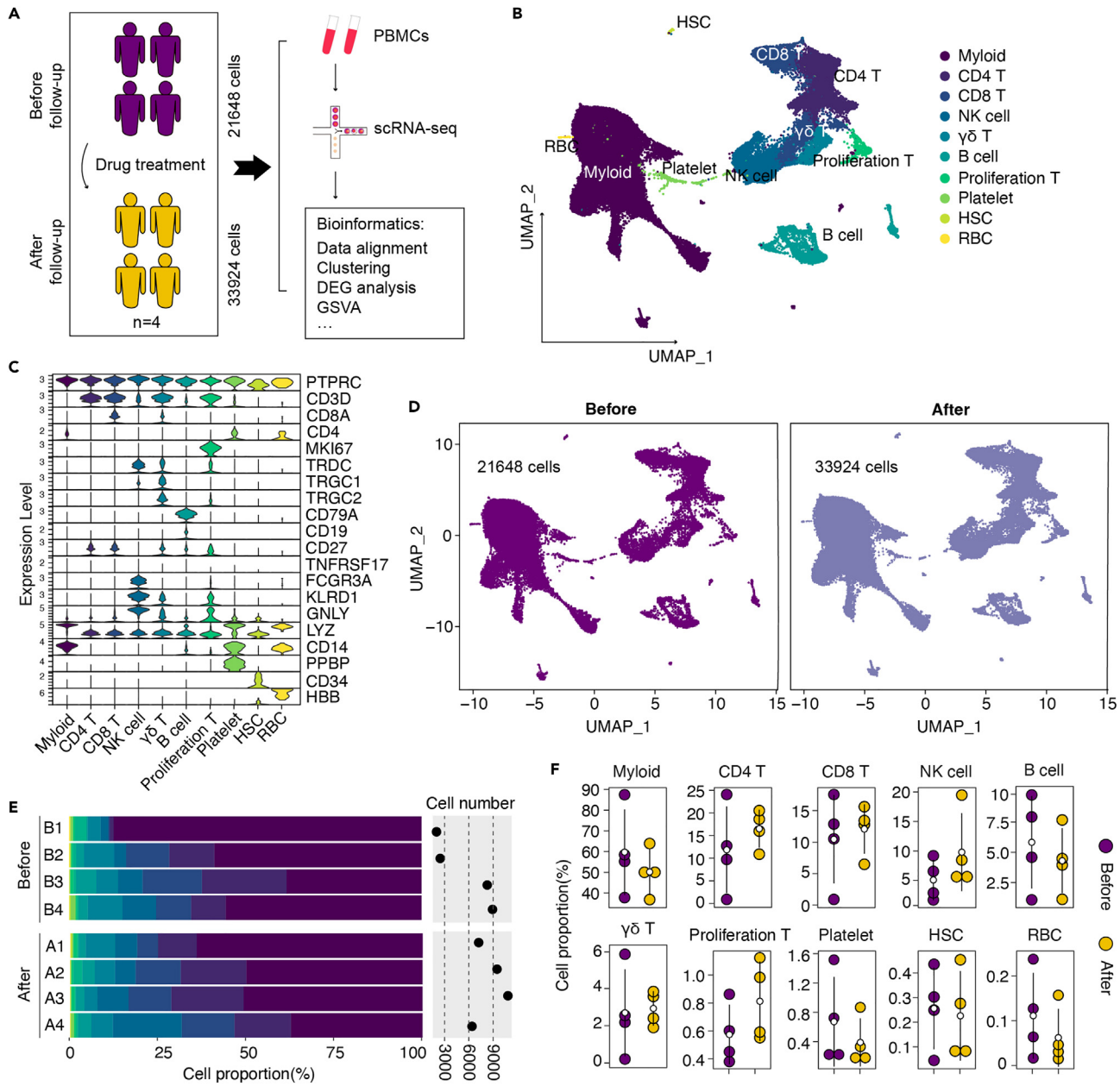

Figure 1. Single-cell RNA-seq profiling of PBMCs from patients with BD

(A) Schematics of the study design.

(B) UMAP plot of the integrated scRNA-seq dataset for ten major cell types.

(C) Violin plot showing the special markers for cell types in Figure 1B.

(D). UMAP plots showing the distribution of PBMCs from patients before and after treatment.

(E) The bar plot showing the proportions of major cell types in PBMCs from diverse individuals. The cell numbers of each dataset from different individuals are listed to the right side.

(F) Dotplot of percentages of each cell types between patients before and after treatment.

memory T cells, CD4 naive T cells, CD8 effector memory T cells, and CD8 central memory T cells mildly increased after drug treatment, while the propotion of NKT cells decreased after treatment (Figure 3C).

To explore the alterations in T and NK cell function, we analyzed differentially expressed genes (DEGs) of CD4 T, CD8 T, and NK cells in patients after drug treatment. Interestingly, we found that expression of

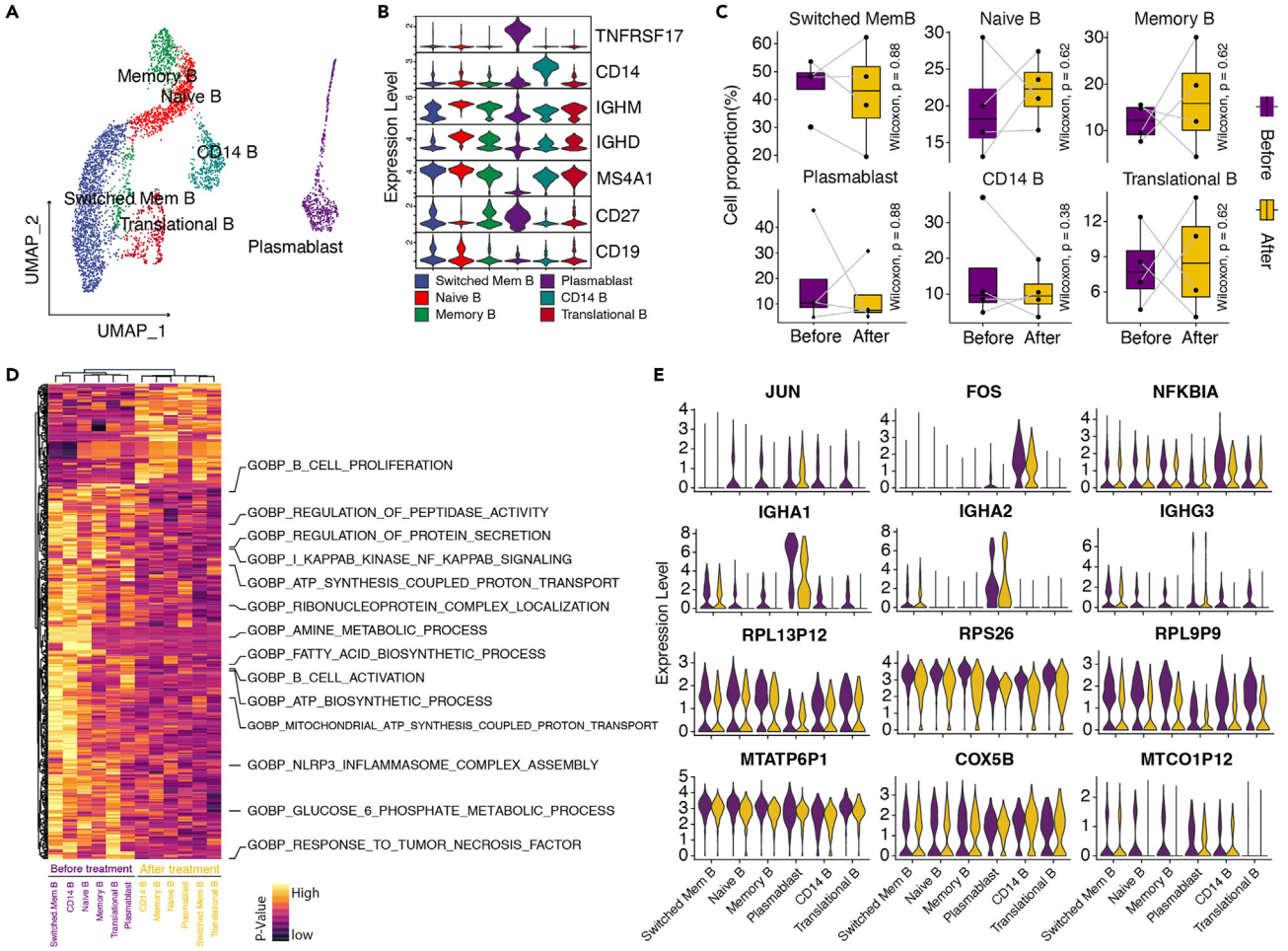

Figure 2. Single-cell analysis reveals abnormal mitochondrial activation of B cells in patients with BD

(A) UMAP plot showing six sub-celltypes for B cells between patients before and after treatment.

(B) Violin plots showing special markers across B cell sub-population.

(C) Boxplots showing the changes of percentages of B cell sub-celltypes between patients before and after treatment.

(D) Gene set variation analysis revealing differences in pathway activities of B cell sub-clusters between patients before and after treatment.

(E) Vlolin plots showing the expressions of inflammation genes (JUN, FOS, and NFKBIA), B cell activation genes (IGHA1, IGHA2, and IGHG3), ribosome proteins genes (RPL13P12, RPS26, and RPL9P9), and mitochondrial respiration genes (MTATP6P1, COX5B, and MTCO1P12) between patients before and after treatment.

common pro-inflammatory cytokines and chemokines in patients before and after drug treatment was similar (Figure 3D). Before treatment, genes involved in mRNA translation were expressed highly in CD4 T and CD8 T cells, while human leukocyte antigen (HLA) genes, which play important role in auto-immune, were expressed highly in NK cells (Figure 3E). A heatmap of DEGs among CD4 T cells, CD8 T cells, and NK cells between pre-treated and post-treated patients was used to analyze genes involved in cytokines and peptides production (Figure 3F). Almost 40% of DEGs in CD4 and CD8 T cells were 40S ribosomal protein (RPS series) and 60S ribosomal protein (RPL series) that were downregulated after drug treatment. Meanwhile, immune-regulated genes such as HLA-DRA, HLA-DRB1, FOS, and JUNB were also downregulated in NK cells (Figure 3F). Moreover, in both CD4 and CD8 T cells, genes involved in ribosomal protein and inflammation were downregulated while genes of the JAK-STAT pathway (BCL2, CISH, IL6ST, and IL7R) were upregulated after drug treatment (Figure 3F). In NK cells, genes involving in cytokine-mediated signaling pathway (CCL3, CCL4, IFR1, IFNGR1, CXCR3R1, CXCR4, and IL18RAP) were upregulated in post-treated patients (Figure 3F). Altogether, these results indicated that current treatment can inhibit global translation levels of T cells and enhance NK cells-mediated immunity in patients with BD.

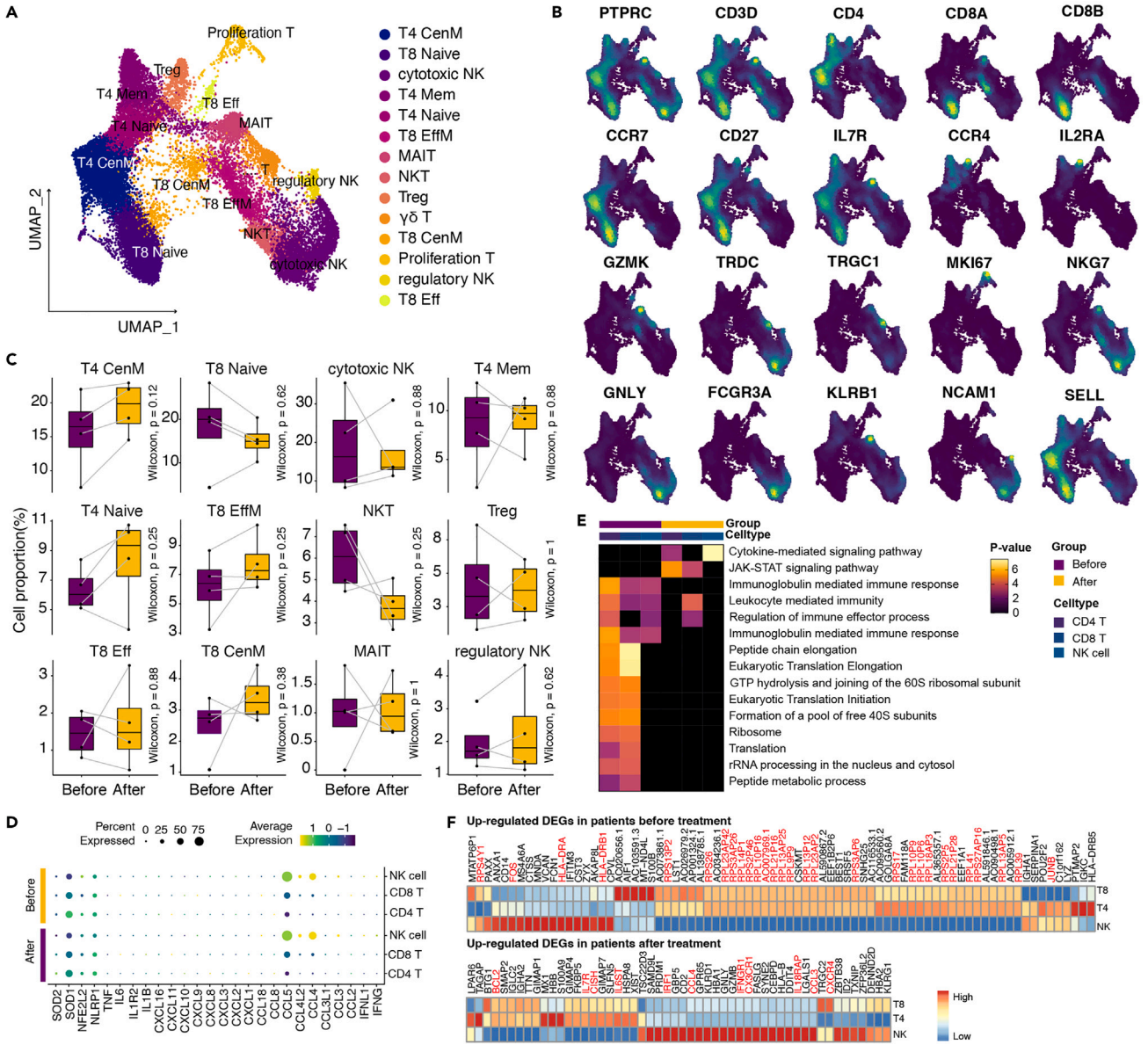

Figure 3. Alteration of T and NK cells in transcriptional profiles in patients with BD

(A) UMAP plot showing six sub-celltypes for T and NK cells between patients before and after treatment.

(B) Feature plot showing special markers across T and NK cell sub-celltypes.

(C) Boxplots showing the changes of percentages of T and NK cell sub-celltypes between patients before and after treatment.

(D) Dotplot showing the expression of major pro-inflammatory genes (superoxide, interleukin cytokine, and chemokine genes) in CD4 T cells, CD8 T cells, and NK cells between patients before and after treatment.

(E) Heatmap showing the main GO terms of highly upregulated genes in CD4 T cells, CD8 T cells, and NK cells between patients before and after treatment.

(F) Heatmap showing the DEGs in CD4 T cells, CD8 T cells, and NK cells between patients before and after treatment.

Decrease in mast cell after treatment

To gain a deeper understanding of the myeloid compartment, we re-clustered myeloid cells and identified 7 cell types, including CD14 monocyte (CD14), CD16 monocytes (FCGR3A), circulating T cell monocyte (CD3D, CD14), cDC (IL3RA), pDC (IL3RA), eosinophil (CLC), and mast cell (FLT3) (Figures 4A and 4B). Interestingly, the proportion of circulating T cell monocyte increased slightly in patients after drug treatment, while the proportion of mast cell decreased (Figure 4C). Previous evidence has suggested that mast cells are typically enriched at the site of inflammation and release pro-inflammatory mediators, exacerbating

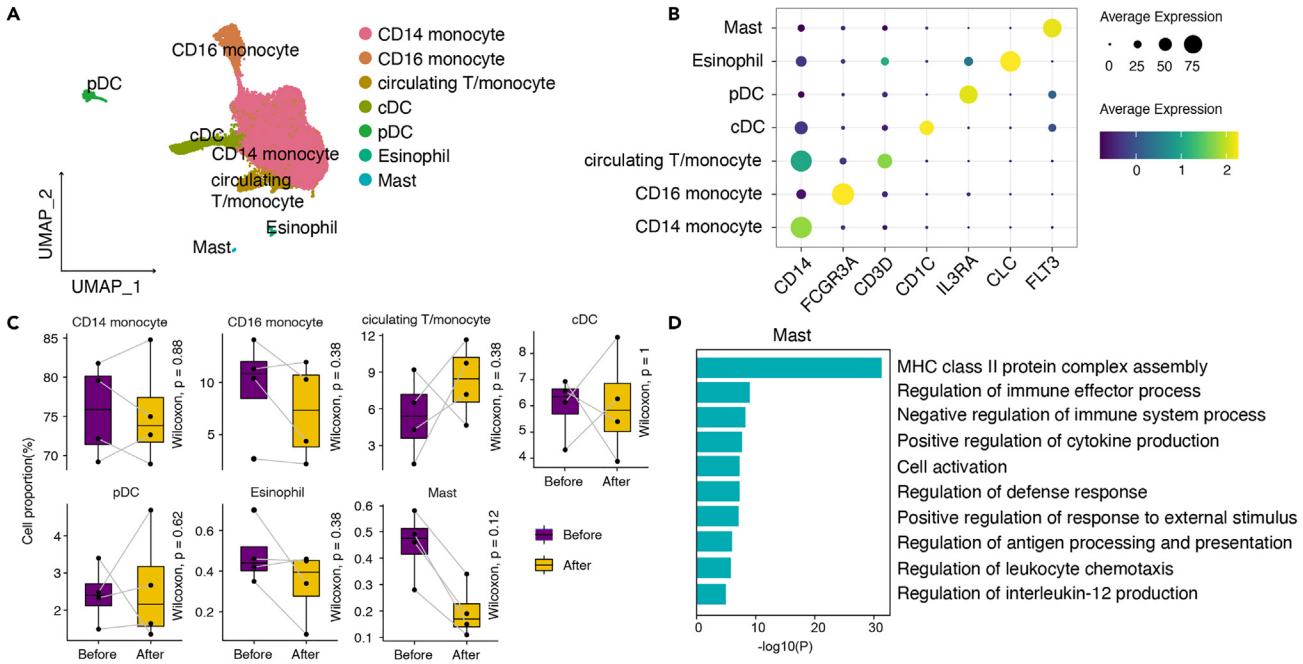

Figure 4. Transcriptional analysis of changes in myeloid cells between patients with BD before and after treatment

(A) UMAP plot showing six sub-celltypes for myloid cells between patients before and after treatment.

(B) Dotplots showing special markers across myloid cells sub-clusters.

(C) Boxplots showing the percentages of myloid cells sub-celltypes between patients before and after treatment.

(D) Gene ontology enrichment analysis of high expression genes in mast cells.

inflammation.[17] Consistently, our data revealed that after drug treatment, many pro-inflammatory-associated genes, such as IL-13 and IL12, were significantly higher in mast cell (Figure 4D, Table S4), indicating that drugs can alleviate inflammation in BD via reducing the number of myeloid cells involved in inflammation response.

Alteration in communications among immune cells

To explore the putative interactions among immune cells in patients, we employed CellChat. We identified 25 unique signaling pathways among 16 cell clusters that were enriched only in pre-treated patients (Figure 5A). Compared to pre-treated patients, the cell-cell communication of immune cells was significantly reduced in post-treated patients, suggesting that immune response was alleviated (Figure 5B). Our results showed that the outgoing signaling pattern from the main myloid cells (CD14 monocyte, CD16 monocyte, and mast cell) decreased significantly in patients after treatment, as well as the incoming signaling pattern targeted CD8 T cell (Figure 5B). 9 out of 25 pathways that were involved in inflammatory and immune response in post-treated patients were inactive, including MCH-I, MCH-II, CD99, ITGB2, ADGRE5, SELPLG, LCK, APP, and CD22 (Figure 5B). Additionally, the main myloid cells (monocyte and mast cells) had a vast expansion of interactions with other cell types through the MCH-I, MCH-II, ITGB2, ADGRE5, and SELPLG signaling pathways in patients before treatment (Figure 5C). Meanwhile, HLA-CD8, HLA-CD4, ITGB2-ICAM, SELPLG-SELL, and ADGRE5-CD55 were the main ligand-receptor pairs that mediated myloid-source-specific communication before drug treatment (Figures 5D and 5E). After treatment, most of the signaling pathways sourced from monocyte were absent, and many immune response pathways from mast cells show a large decrease (Figures 5D and 5E). Together, our data suggest that drugs can significantly reduce cell-cell communication sourced from myloid cells, highlighting their essential roles in mediating inflammation.

DISCUSSION

By utilizing single-cell technologies, we were able to detect the immune alterations in specific cell populations during drug treatment at a single-cell resolution. This approach allowed us to explore the dynamics

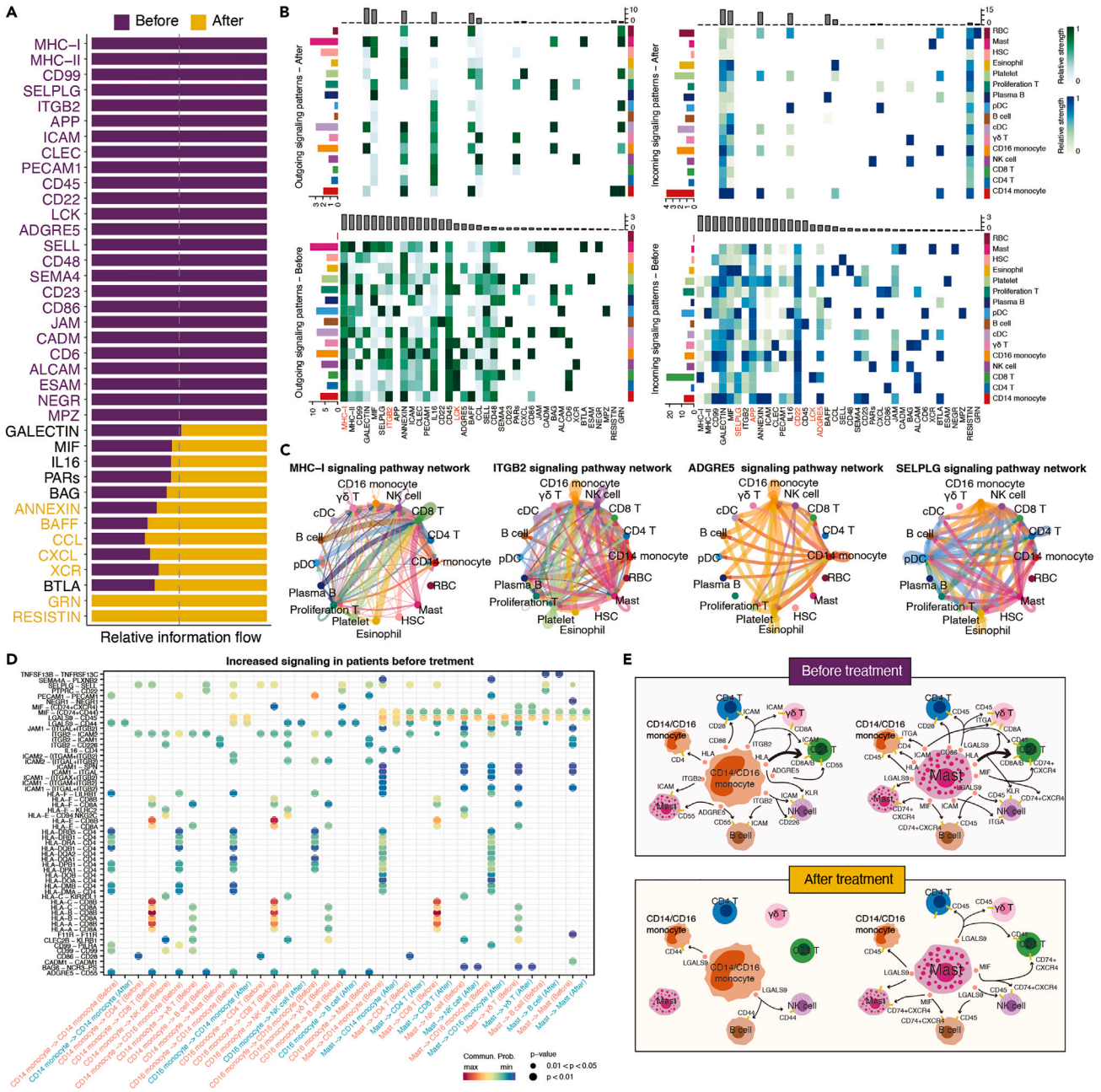

Figure 5. CellChat analysis of the communications between PBMCs in patients with BD

(A) The significant signaling pathways were ranked based on their differences of overall information flow within the inferred networks between patients before and after treatment.

(B) The heatmap showing the comparison of outgoing signaling patterns of secreting cells and incoming signaling patterns of recepting cells between patients before and after treatment.

(C) Circos plot showing four high variable intercellular communication networks across major cell types for patients before and after treatment.

(D) Comparison of the significant ligand-receptor pairs between patients before and after treatment, which contribute to the signaling from CD14 monocyte, CD16 monocyte, and mast cells to major cell types. P-values were represented by the dot size and communication probabilities were showed as dot color. Empty space means the communication probability is zero.

(E) Schematic overview of the alternation in cell communications between patietns before and after treatment.

of cell-cell communication before and after treatment, which was not feasible with traditional bulk sequencing methods. Our study assessed a total of 55,572 cells using scRNA-seq and identified 10 main cell clusters and 26 subclusters. We observed that the cell proportions of diverse clusters showed similar tendencies between untreated and treated patients, with the exception of two inflammation-related cells, mast cells, which tended to decrease after treatment. We also found that the expression of inflammation-related genes in B cells decreased after treatment, along with a decrease of mast and eosinophil cell populations. Combining treatment inhibited mRNA translation in both B cells and T cells, which may contribute to immune cell activation and inflammation, but elevated NK-mediated cytokine immunity. Additionally, global cell-cell communication among immune cells was remarkably reduced, and multiple immune response and inflammation pathways were eliminated after treatment.

As previously described, immune dysfunction is associated with a high risk of BD.[18] Autoimmune disorders are the most common representation of immune disorders, characterized by the immune system's recognition disorder, which may mistakenly remove host tissues and result in a large-scale inflammatory response throughout the body.[19] Once the inflammatory response is triggered, pro-inflammatory factors are released, eventually entering the CNS. Ribosomes are crucial in biology processes and contribute to the innate immune response.[20] Besides serving as the center of post-transcription translation, previous evidence has found that numerous ribosomal proteins can contribute to virus transcripts translation and promote inflammatory responses.[21] Furthermore, the expression of translation factors and ribosomal proteins, such as eIF2B, eIF3, eIF4, eEF1, and eEF2, has been reported aberrantly high in schizophrenia-patients-derived induced pluripotent stem cells.[22] Dysfunction in protein synthesis, such as eIF2α signaling pathway, was associated with BD.[23] Our study found that both B cell and T cell clusters, particularly in T cells, expressed primarily ribosomal protein genes, including RPS2, RPS3, RPS4, RPS17, RPS26, RPL4, RPL9, RPS17, RPL10, RPL13, RPL14, RPL21, RPL23, and RPL39, in patients with BD. After drugs treatment, all these ribosomal genes were downregulated, suggesting us that targeting ribosomal proteins may be an important way to alleviate inflammation and immune dysfunction in patients with BD.

It is well known that mitochondria are the center of cellular metabolism and generate most of the ATP to provide enough energy for normal life activities.[24] In the progression of immune responses, immune cells can transform from metabolic quiescence to active status, characterized by elevating levels of ATP.[25] What's more, inflammatory responses can be activated by mitochondria via enhancing NLRP3 assembly.[26] Our results revealed that genes involved in mitochondrial ATP generation, such as MTATP6P1 and MTCO1P12, and B cell activation genes, IGHA1, IGHA2, and IGHG3, were highly expressed in pre-treated patients with BD. We also found that genes involved in inflammation-related pathways, such as NLRP3 assembly, NFKBA signaling, and response to tumor necrosis factors, were downregulated after treatment. These findings suggest that drugs can effectively reduce energy metabolism in B cells, thereby contributing to alleviate inflammation responses in patients with BD. In contrast, we found that many pro-inflammation cytokine genes, such as CCL3, CCL4, and CXCR4, were highly expressed in patients after treatment. We speculated that rising immune responses in NK cells may be associated with the clearance of some pro-inflammatory cells, such as mast cells and eosinophils.

Cell-cell communication among immune cells may play an essential role in the inflammation response in patients with BD. Our analysis of cell interactions showed a surge in both outgoing and incoming signaling pathways in patients with BD. In pre-treated patients, the signaling pathways involving ITGB2-ICAM1, CD97(ADGRE5)-CD55, and PSGL-1(SELPLG)-SELL, which are derived from monocytes, were enhanced, and contributed significantly to the inflammatory response. Adhesion receptor ICAM-1 can recruit leukocytes from circulation to sites of inflammation to drive the inflammation response.[27] CD97, a member of the adhesion G protein-coupled receptor family, is highly expressed on inflammatory cells.[28] PSGL-1, which can express on the surface of myeloid cells, mediates the inflammatory response by promoting leukocyte migration into inflamed locations.[29] Furthermore, our results showed that the MHC-I signaling pathway targeting CD8 T cells was significantly reduced in patients after treatment. The MHC-I pathway participates in immune regulation by activating CD8 T cells,[30] and excessive and continuous antigen stimulation on T cells can lead to T cell exhaustion and immune dysfunction.[31] Therefore, we suggested that the MHC-I pathway is one of the most important targets to alleviate severe inflammation in patients with BD.

In conclusion, we used scRNA-seq to analyze the immune landscape in patients with BD after quetiapine and valproate treatment. Our findings indicate that this drug treatment effectively reduces immune

response among patients with BD by comprehensively reducing communication levels between immune cells, without significantly affecting the number of immune cells. Moreover, our results suggest that a combination of drugs can also inhibit the expression of ribosomal protein-related genes on both B and T lymphocytes and mitochondrial ATP synthesis on B cells of patients with BD. Unlike B and T cells, NK-mediated immunity seems to be elevated after treatment, which may contribute to abnormal cell clearance. Based on our current findings, we believe that scRNA-seq technology holds great promise for managing BD, including developing treatment strategies and improving patient outcomes. Our study has confirmed the crucial role of anti-inflammation in the treatment of patietns with BD, and we have also identified a potential avenue for alleviating disease progression by inhibiting the function of ribosomes in B and T cells. Additionally, our results have highlighted the significance of the MHC-I pathway in exacerbating inflammation during BD progression, which could be a key target for future development. Overall, our research sheds light on how current treatments can reduce inflammation in patietns wtih BD and provides valuable data for enhancing the diagnosis and treatment of this condition.

Limitations of the study

Despite our analysis of immune dynamics in patients before and after drug treatment, our study has some notable limitations. The sample size in our study was relatively small compared to cohort study.[32,33] Moreover, the samples of drug-treated group were collected only one month after treatment, and a long-term observation of drug effects is needed. In addition, our study focused only on immunology and lacked evidence in neurobiology, primarily due to the challenges of obtaining brain tissues legally and efficiently. Further independent experiments, such as Western blotting and other functional validations, are necessary to explore thoroughly the immune-neuron crosstalk between pre- and post-treatment.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107057.

ACKNOWLEDGMENTS

We gratefully thank many of our colleagues for their important and helpful comments. This study was supported by grants from the National Natural Science Foundation of China (Grant No.81971258), Key-Area Research and Development Program of Guangdong Province (Grant No. 2018B030334001), National Natural Science Foundation of China (Grant No.82271571), and Scientific and Technological Innovation Program of High-tech Industry of Hunan Province (Grant No. 2020GK4082).

AUTHOR CONTRIBUTIONS

L.Q. conceived and designed analyses, performed analyses, and wrote the paper. Y.Q. collected the specimens and conceived analysis design. S.L., N.Y., C.L., Z.T., X.X., C.Z., Y.Y., J.H., C.W., and J.C. helped collect

the specimens. S.L. and B.L. helped data analyses. H.W. designed analyses, provided the patients' clinical information, and revised the manuscript. Z.X. designed analyses and contributed to manuscript preparation. B.L. designed analyses, collected the specimens, and wrote the paper.

DECLARATION OF INTERESTS

The authors confirm that there are no conflicts of interest.

Received: December 19, 2022
Revised: March 22, 2023
Accepted: June 1, 2023
Published: June 7, 2023

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
PBMCs from individuals pre- and post-treatment. See Table S1	Institutional Review Board of Xiangya Second Hospital, Central South University	BDPBMC-YP
Chemicals, peptides, and recombinant proteins		
1× PBS	BioSharp	Car#BL302A
Ficoll solution	Solarbio	Car#P8900
1× RBC Lysis Buffer	Invitrogen	Car#00-4333-57
Critical commercial assays		
GEXSCOPE® Single Cell RNA Library Kit Cell (Matrix) V2	Singleron	Car#4180031
Deposited data		
Raw and analyzed data	This paper	NGDC: HRA002970
Software and algorithms		
Graphpad Prism	https://www.graphpad.com/features	version 9.5.0
CellRanger	https://support.10xgenomics.com/	version 6.1.1
R base	https://cran.r-project.org/	version 4.0.3
Seurat	https://satijalab.org/seurat/	version 4.1.0
Metascape	http://metascape.org/	version3.5.20230101
GSVA	https://bioconductor.org/packages/GSVA	version 1.36.3
venn	https://github.com/dusadrian/venn	version 1.10
ggsignif	https://const-ae.github.io/ggsignif/	version 0.6.0
ggplot2	https://ggplot2.tidyverse.org	version 3.4.0
CellChat	http://www.cellchat.org/	version 1.1.3
pheatmap	https://cran.r-project.org/	version 1.0.12

RESOURCE AVAILABILITY

Lead contact

Additional information and requests for resources should be directed to the Lead contact, Bo Lv (lvbo@tongji.edu.cn)

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Single-cell RNA sequencing data generated in this paper have been deposited in the National Genomics Data Center under accession number HRA002970.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ethics statement

Our study followed the ethical standards of the Declaration of Helsinki Ethical approval was obtained from the Institutional Review Board of Xiangya Second Hospital, Central South University (BDPBMC-YP). All participating patients provided written informed consent.

Patient recruitment and drug treatment

Four patients with BD in depressed episodes (2 females and 2 males) were recruited at Second Xiangya Hospital to conduct research from January to February 2022 (Table S1). In our study, each patient received 100mg quetiapine and 1000mg valproate daily. For pre-treatment group, we isolated PBMCs from each patient before drug treatment and we collected samples after one-month treating for post-treatment group. The clinical characteristics of patients before and after treatment are presented in Table S2. All individuals in both pre and post treatment groups are paired.

METHOD DETAILS

Peripheral blood mononuclear cell (PBMC) isolation

The peripheral blood mononuclear cells (PBMCs) were isolated from whole blood according to standard protocols.[34] Firstly, blood cells were roughly isolated from EDTA anticoagulated blood collection tube by centrifuging at 500×g for 10 minutes. After resuspending cellular pellets in 1× PBS (BioSharp, BL302A), PBMCs were then isolated by centrifuging at 500×g for 30 minutes on Ficoll solution (Solarbio, P8900). After discarding the supernatant, erythrocytes in single cell suspensions were lysed by using 1× RBC Lysis Buffer (Invitrogen, 00-4333-57) at room temperature for 8 minutes. Cell viability was assessed by trypan blue staining, and cell viability exceeded 90% were performed on the single cell RNA sequencing.

Single-cell suspension preparation and single cell RNA sequencing

Single-cell suspensions with a concentration of 1×10^5 cells/mL in PBS were loaded into microfluidic devices. Subsequently, single-cell RNA sequencing libraries were constructed using the Singleron GEXSCOPE® Single Cell RNA Library Kit Cell (Matrix) V2 (Singleron Biotechnologies, China). The cDNA libraries were diluted to 5 nM and then subjected to sequencing on the Illumina NovaSeq 6000 platform.

Single-cell RNA data processing

The transcripts were filtered and aligned to the human reference genome h38 by using CellRanger software (version 6.1.1) with the default parameters. After achieving clean data, downstream analysis was mainly performed on Seurat R package (version 4.1.0).[35] Cells those genes detected less than 1000 were filtered, raw counts of left cells were normalized with the default parameters. Top 2000 variable genes were used to perform principal component analysis (PCA). Batch effect was reduced to reasonable degree and data from different batches was integrated to one Seurat object. The data were scaled to suitable degree and made dimensional reduction by using uniform manifold approximation and projection (UMAP). Then, a nearest-neighbor graph using 50 dimensions of the PCA reduction was calculated, followed by clustering with a resolution of 0.8. Different expression genes (DEGs) were calculated by using the MAST method of 'FindMarkers' function. Average logarithmic fold changes over 0.5 were input to perform gene ontology enrichment analysis was analysis by online tools of Metascape (http://metascape.org/).[36] Linage scores were calculated by calculating as the sum of logarithm of cpms among selected genes.[37] Venn diagrams were illustrated by venn R packages (version 1.10). Statistical analyses were performed by ggsignif R packages (version 0.6.0).

Gene set variation analysis (GSVA)

GSVA analyses were performed on the 7481 GO gene sets annotated in the Molecular Signatures Database by using GSVA packages (version 1.36.3) with default parameters.[38] The output results were visualized with heatmap by using pheatmap R packages (version 1.0.12).

Receptor and ligand interactions analysis

Interactions between main cell types were evaluated by CellChat R packages (version 1.1.3).[39] To identify potential intercellular interactions, we concentrated on differentially expressed ligands and receptors in myloid cells, CD4 T cells, CD8 T cells, proliferation T cells, NK cells, γδ T cell, B cells, plasmablast and platelet. The entire paired datasets were selected to perform the cell communications.

QUANTIFICATION AND STATISTICAL ANALYSIS

The bioinformatics data were statistically analyzed using an unpaired, two-tailed Mann-Whitney U tests with R language. Clinical data in Table S2 were statistically analyzed by using paired t-test with Graphpad Prism (Version 9.5.0). All clinical data for performing paired t-test were passed the normality and

lognormality tests. Unless otherwise specified, all clinical data are presented as mean ± standard error of the mean (SEM). For all analyses, $P < 0.05$ was considered statistically significant.

ADDITIONAL RESOURCES

This study has been registered at Chinese Clinical Trial Registration (www.chictr.org.cn) and clinical registry number is ChiCTR1900021379.