ORIGINAL RESEARCH

Mendelian Randomization and Transcriptomic Analysis Reveal the Protective Role of NKT Cells in Sepsis

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Background: Sepsis is a life-threatening clinical syndrome caused by dysregulated host response to infection. The mechanism underlying sepsis-induced immune dysfunction remains poorly understood. Natural killer T (NKT) cells are cytotoxic lymphocytes that bridge the innate and adaptive immune systems, the role of NKT cells in sepsis is not entirely understood, and NKT cell cluster differences in sepsis remain unexplored.

Methods: Mendelian randomization (MR) analyses were first conducted to investigate the causal relationship between side scatter area (SSC-A) on NKT cells and 28-day mortality of septic patients. A prospective and observational study was conducted to validate the relationship between the percentage of NKT cells and 28-day mortality of sepsis. Then, the single-cell RNA sequencing (scRNA-seq) data of peripheral blood mononuclear cells (PBMCs) from healthy controls and septic patients were profiled.

Results: MR analyses first revealed the protective roles of NKT cells in the 28-day mortality of sepsis. Then, 115 septic patients were enrolled. NKT percentage was significantly higher in survivors (n = 84) compared to non-survivors (n = 31) (%, 5.00 ± 3.46 vs 2.18 ± 1.93, P < 0.0001). Patients with lower levels of NKT cells exhibited a significantly increased risk of 28-day mortality. According to scRNA-seq analysis, NKT cell clusters exhibited multiple distinctive characteristics, including a distinguishing cluster defined as FOS⁺NKT cells, which showed a significant decrease in sepsis. Pseudo-time analysis showed that FOS⁺NKT cells were characterized by upregulated expression of crucial functional genes such as *GZMA* and *CCL4*. CellChat revealed that interactions between FOS⁺NKT cells and adaptive immune cells including B cells and T cells were decreased in sepsis compared to healthy controls.

Conclusion: Our findings indicate that NKT cells may protect against sepsis, and their percentage can predict 28-day mortality. Additionally, we discovered a unique FOS⁺NKT subtype crucial in sepsis immune response, offering novel insights into its immunopathogenesis.

Keywords: natural killer T cells, sepsis, outcome, Mendelian randomization

Introduction

Sepsis, a significant global health issue, annually impacts millions worldwide, with an estimated 48.9 million cases and 11 million related deaths, constituting about 1/5 of all global fatalities.[1] Recently, studies assessing the immunophenotypes of septic patients have shed light on the immune dysregulation that occurs during sepsis.[2] However, the mechanism underlying sepsis-induced immune dysfunction remains poorly understood.

Natural killer T (NKT) cells are a type of cytotoxic lymphocytes that bridge the innate and adaptive immune systems. They recognize glycolipid antigens presented by the CD1d molecule on antigen-presenting cells.[3] Once activated, NKT cells rapidly produce large amounts of cytokines like interferon (IFN)-γ and interleukin (IL)-4, which stimulates downstream activation of other cell types like NK cells, T cells, B cells, and dendritic cells.[4] Mouse sepsis model by

streptococcus pneumoniae revealed inhibition of NKT cell activation led to higher bacterial load.[5] However, mice lacking invariant NKT cells (Jalpha18(-/-)) had both reduced mortality in response to cecal ligation and puncture (CLP)-induced sepsis and a diminished systemic inflammatory response.[6] Thus, the role of NKT cells in sepsis is complex, and our knowledge of which remains incomplete. Efforts are needed to fully elucidate and precisely define the contributions of NKT cells to the development of sepsis.

Mendelian randomization (MR) is a method used in epidemiology and genetics to assess causal relationships between risk factors and disease outcomes. It can provide a way to overcome possible limitations of observational studies and identify potential causal relationships between molecular traits and phenotypes.[7] Compared to randomized controlled trials (RCTs), MRs can provide a more cost-effective, rapid, and ethical assessment of the long-term impacts of exposure on outcomes.[8] Wang et al, using a comprehensive MR analysis, demonstrated the causal association between immune phenotypes and schizophrenia, highlighting the complex pattern of interactions between the immune system and schizophrenia.[9] Since findings concerning the associations between NKT cells and sepsis are inconsistent, in this study, a two-sample MR analysis was first performed to determine the causal association between NKT cell signatures and sepsis, particularly those with worse outcomes. Then, the relationship between the percentage of NKT cells and the outcome of patients with sepsis was validated by flow cytometry.

Recent advancements in biotechnology, along with extensive research on NKT cells, have facilitated a deeper classification of NKT cell subtypes using single-cell RNA sequencing (scRNA-seq). Unlike traditional bulk RNA sequencing methods, which provide average expression levels across a population of cells, scRNA-seq allows researchers to capture the heterogeneity present within cell populations by examining gene expression at the single-cell level. Thus, the distinct functional roles of these NKT cell subtypes in disease processes are being progressively elucidated.[10,11] Wang et al found that a type of circulating CD160^{hi}NKT cells was decreased in patients with carbapenem-resistant Klebsiella pneumonia (CRKP) infection, which may facilitate bacterial immune evasion and indicate suppressed host–immune responses against CRKP.[12] However, the function and heterogeneity of NKT cells in sepsis are unclear.

In this study, we first estimate the association between NKT cell traits and sepsis with genome-wide association study (GWAS) summary data, then we enrolled septic patients to validate the relationship between the percentage of NKT cells and 28-day mortality. At last, we leveraged single-cell RNA sequencing (scRNA-seq) to comprehensively profile transcriptional diversity within NKT cells. We illustrated NKT function and heterogeneity in sepsis and identified a specific NKT cluster and its role in the progression of sepsis.

Methods
GWAS Data Sources
The GWAS summary statistics for each immune trait are publicly available in the GWAS catalog (accession number from GCST90002084).[13] The GWAS summary statistics for sepsis (N_{case}=1896, $N_{control}$=484,588) are based on data published by the UK Biobank consortium. These statistics can be accessed through the IEU Open GWAS website with the identifier ieu-b-5086 (for 28-day mortality).

Selection of instrumental variables (IVs)
By published research,[9] the significance level of IVs for each immune trait was set to 1×10^{-5}. The clumping procedure in PLINK software was used to prune single-nucleotide polymorphisms (SNP) (linkage disequilibrium [LD] r^2 threshold < 0.1 within 500 kb distance).[14]

Mendelian Randomization
All MR analyses were performed in R 4.3.1 software using the "TwoSampleMR" package (version 0.9.0). Inverse variance weighting (IVW),[15] weighted medians,[16] multiplicative random effects (MR-Egger), simple mode, and weighted mode were performed to evaluate the causal association between NKT cell traits and sepsis. IVW, the most commonly used and efficient method, was used as the primary analysis for this study, due to its highest power. If there is a violation of the assumption of no pleiotropy, MR-Egger can be a more reliable alternative. Depending on the specific research question and the available data,

the weighted median, simple mode, and weighted mode each have their strengths and weaknesses and can be helpful in their ways. We conducted horizontal pleiotropy analysis using MR-Egger regression. Heterogeneity in the effect size of SNP-specific causal effects during two-sample MR was examined using Cochran's Q-test.[17] Finally, we conducted a leave-one-out sensitivity analysis to assess the impact of individual SNP on the overall estimates.

Human Patients

Blood samples were obtained from sepsis patients within 24 hours of meeting the diagnostic criteria of sepsis. Sepsis was diagnosed based on the sepsis-3 criteria, which involves patients with confirmed or suspected infection and a sudden increase in the total Sequential Organ Failure Assessment (SOFA) score by ≥2 points. Infection was identified using the International Classification of Diseases, 10th Edition (ICD-10) code.[18] Exclusion of those who are younger than 18 years, pregnant, or with malignancy, chronic viral infection, such as HIV, hepatitis B or C; autoimmunity, corticosteroid use, or consent could not be obtained.

Flow cytometry

Cells were stained with anti-CD45-Brilliant Violet 570™ (BioLegend), anti-CD3-FITC (BioLegend), and anti-CD56-PE (BioLegend). Samples were collected on a NovoCyteD3000 flow cytometer. Data were further analyzed by FlowJo software (9.9.6 FlowJo., LLC).

Single-Cell RNA-Seq Analysis

We retrieved single-cell RNA sequencing datasets for patients with sepsis from the Gene Expression Omnibus (GEO) database, using the accession number GSE151263. Additionally, scRNA-seq data for healthy control (HC) subjects were acquired from the GEO database, utilizing the accession number GSE157007. We utilized the R package Seurat for the analysis of scRNA-seq data, focusing on identifying distinct cell types and exploring variations in immune cells.[19] To ensure data quality, we applied specific filtering criteria for cells: (1) Ensuring that the number of expressed RNA features per cell (nFeature_RNA) was between 300 and 2000, and (2) Limiting the percentage of mitochondrial genes to less than 10%. Normalization, dimensionality reduction, and clustering of scRNA-seq data were then performed. Uniform Manifold Approximation and Projection (UMAP) was used to visualize the clusters. The "FindMarkers" function was used to identify differentially expressed genes (DEGs) between two cell groups. Cell-cell communication analysis was conducted in CellChat (v1.6.1) software. To re-cluster the NKT cells, the "FindClusters" function was applied, resulting in the formation of 6 new NKT cell clusters. To unravel the developmental trajectories of NKT cell clusters, we conducted a pseudo-time analysis employing the Monocle 2 algorithm (v2.24.0) during Pseudo-time Analysis.[20] To assess transcription factor activity at the individual cell level, we employed the SCENIC algorithm.[21] Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were used to evaluate the biological functions and signaling pathways associated with the identified genes.[22,23]
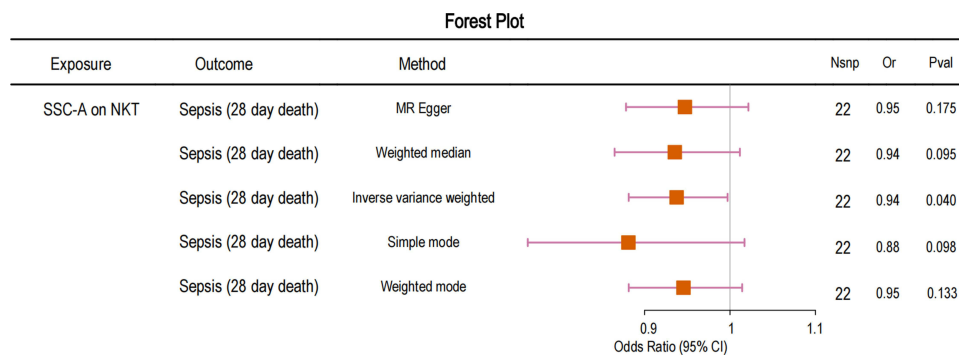
Statistics

The data were analyzed by GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Continuous variables between the two groups were compared using the student's t-test or the Mann-Whitney U test. Categorical variables were compared using the Chi-squared test or Fisher's exact test. Receiver operating curve (ROC) analysis, along with the Youden index, was utilized to establish the optimal cutoff value. Additionally, survival analysis was conducted using the Kaplan–Meier curve (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Result

NKT cells had a significant causal effect on the 28-day mortality of sepsis

Upon thorough evaluation of multiple MR techniques, IVW results delineate a significant relation between SSC-A on NKT cells and the 28-day mortality rate of sepsis, with an OR of 0.938 (95% CI: 0.881–0.997, P = 0.040) (Figure 1). The robustness of the causal associations observed was proved by the heterogeneity analysis (Table S1). The intercept of MR-

Forest Plot

Exposure	Outcome	Method		Nsnp	Or	Pval
SSC-A on NKT	Sepsis (28 day death)	MR Egger		22	0.95	0.175
	Sepsis (28 day death)	Weighted median		22	0.94	0.095
	Sepsis (28 day death)	Inverse variance weighted		22	0.94	0.040
	Sepsis (28 day death)	Simple mode		22	0.88	0.098
	Sepsis (28 day death)	Weighted mode		22	0.95	0.133

0.9   1   1.1
Odds Ratio (95% CI)

Figure 1 Forest plot to visualize the causal effect of SSC-A on NKT on the risk of sepsis 28-day mortality.

Egger ruled out the possibility of horizontal pleiotropy (Table S2). Scatter plots and funnel plots both indicated the stability of the results (Figure S1A-B). The study also conducted the leave-one-out analysis to eliminate the impact of individual SNPs on the overall causal estimate by the stepwise removal of each SNP and repeating the MR analyses. After removing each SNP, the leave-one-out analysis showed a relatively stable outcome (Figure S1C). Thus, SSC-A on NKT cells had significant causal effects on the 28-day mortality of sepsis, qualifying it as a protective determinant on the 28-day mortality rate of sepsis.

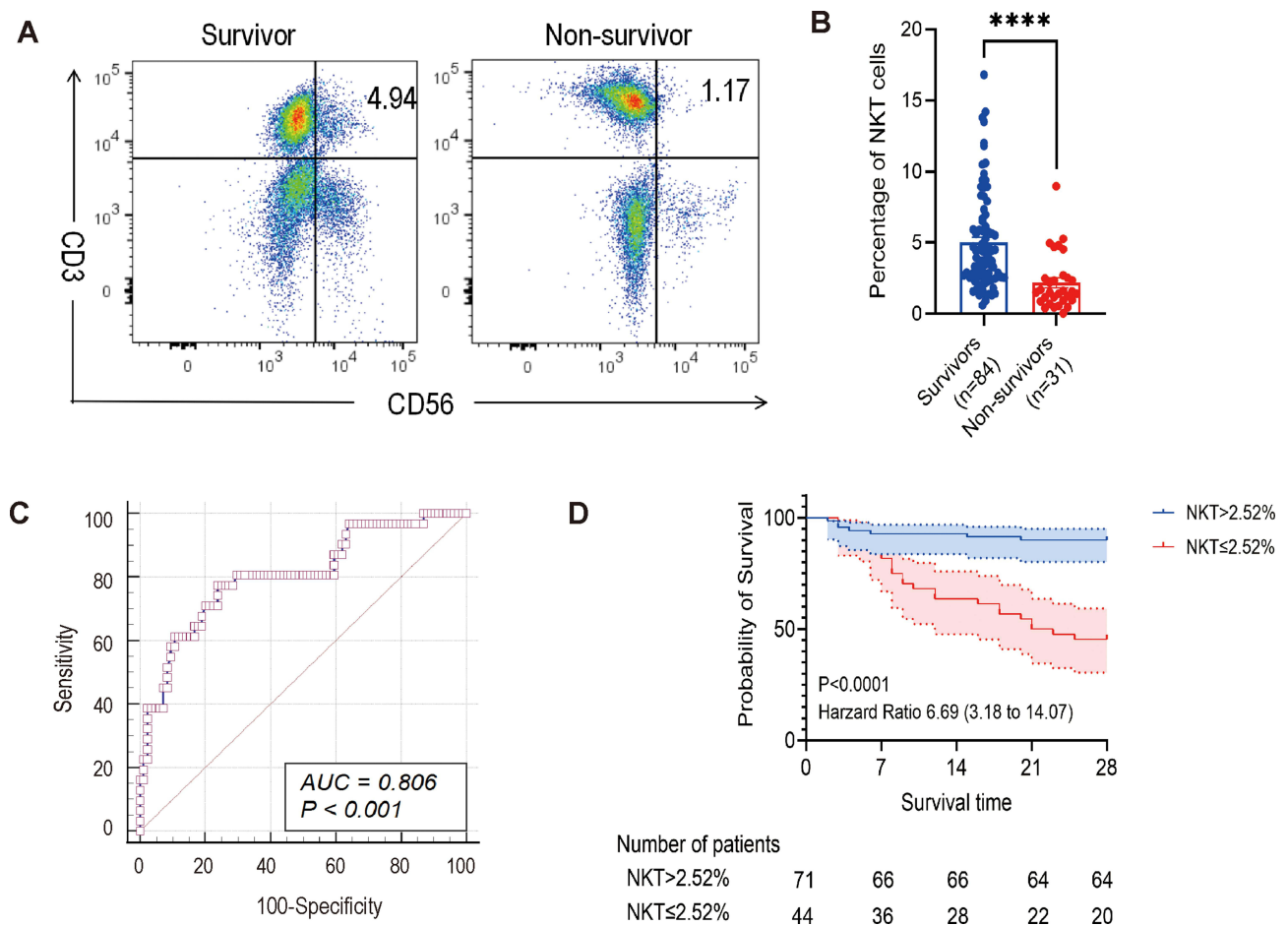## Percentage of NKT Cells Was Associated with 28-Day Mortality of Sepsis

To investigate the relationship between NKT cells and the severity of sepsis, we conducted a prospective and observational study enrolling 115 septic patients, 84 of whom survived and 31 did not. There were no significant differences in age, gender, and body mass index (BMI) between survivors and non-survivors. Non-survivors had higher SOFA score (11 ± 5 vs 8 ± 4, $P = 0.004$), and Acute Physiology and Chronic Health Evaluation (APACHE) II score (25 ± 9 vs 19 ± 8, $P = 0.001$) compared to survivors (Table 1).

The percentage of NKT cells in 28-day survivors was significantly higher when compared to non-survivors (%, 5.00 ± 3.46 vs 2.18 ± 1.93, $P < 0.0001$) (Figure 2A-B). Subsequently, a ROC curve was employed to assess the ability of NKT

Table 1 Clinical Characteristics of the Patients According to 28-Day Survival

	Survivors (n=84)	Non-survivors (n=31)	p
Age, (year, mean±SD)	67±15	69±14	0.594
Male, n (%)	51 (0.61)	24 (0.77)	0.123
BMI, (kg/m², mean±SD)	24.44±4.96	23.09±3.46	0.167
Source of Infection, n (%)			0.097
Lung	25 (0.30)	16 (0.52)	
Abdomen	42 (0.50)	10 (0.33)	
Urinary	9 (0.11)	1 (0.03)	
Other	8 (0.10)	4 (0.13)	
Hypertension, n (%)	40 (0.48)	14 (0.45)	0.8365
Coronary heart disease, n (%)	14 (0.17)	5 (0.16)	> 0.999
COPD, n (%)	3 (0.04)	0 (0)	0.562
Diabetes, n (%)	28 (0.33)	16 (0.52)	0.086
White cells count [10^9/L, M (IQR)]	11.87 (8.45–18.50)	13.08 (8.62–16.83)	0.688
C-reactive protein [mg/mL, M (IQR)]	131.50 (84.10–204.80)	157.60 (120.20–248.30)	0.240
PCT [ng/mL, M (IQR)]	5.95 (1.32–18.94)	4.96 (1.48–26.65)	0.859
SOFA score, mean±SD	8±4	11±5	0.004
APACHE II score, mean±SD	19±8	25±9	0.001

Abbreviation: BMI, Body Mass Index; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; COPD, Chronic Obstructive Pulmonary Disease; PCT, Procalcitonin.

Figure 2 The percentage of NKT cells was associated with 28-day mortality of sepsis. (**A**) Representative flow cytometry graphs of NKT cells. (**B**) Percentage of NKT cells between 28-day non-survivors (n=31) and 28-day survivors (n=84). (**C**) ROC analysis of the percentage of NKT cells predicting 28-day mortality in septic patients. (**D**) Kaplan-Meier analysis of survival probability in septic patients with a percentage of NKT cells >2.52% vs ≤2.52%. ****p<0.0001.

cell percentage to predict 28-day mortality in septic patients. The results showed an AuROC of 0.806 (95% CI: 0.722–0.874; P < 0.001) (Figure 2C). Using J statistics, a cutoff value of 2.52% was determined, providing a sensitivity of 77.42% and a specificity of 76.19%. Notably, patients with lower levels of NKT cells exhibited a significantly increased risk of 28-day mortality (OR, 6.69; 95% CI, 3.18–14.07; P < 0.0001) (Figure 2D).

Impaired Function of NKT Cells in Sepsis

To investigate NKT cell function in sepsis, we isolated PBMCs from 7 septic patients and 3 controls. Using UMAP, we identified 6 distinct cellular populations (Figure 3A), including myeloid cells (*S100A9* and *LYZ*), T cells (*CD3E, CD3G*, and *CD3D*), B cells (*CD19, CD79A*, and *CD79B*), NK cells (*KLRD1, GNLY*, and *NKG7*), NKT cells (*CD3D, CD3E, CD3G, KLRD1* and *KLRB1*), and platelets (*ITGA2B* and *PPBP*) (Figure 3B). Bar plots illustrated the distribution of these populations in each group (Figure 3C). Statistical analysis revealed a significant decrease in NKT cell frequency in sepsis compared to controls (Figure 3D), suggesting a potential role of reduced NKT cells in sepsis-induced dysfunction.

GO analysis was conducted to investigate the gene function of NKT cells in sepsis (Figure 3E). The analysis revealed involvement in mononuclear cell differentiation, positive regulation of epithelial cell proliferation, and integrated stress response signaling. Additionally, KEGG pathway analysis identified enrichment in pathways such as the IL-17 signaling pathway, TNF signaling pathway, and B cell receptor signaling pathway (Figure 3F).

To investigate cell-cell communications between NKT cells and other immune cells, Cellchat was utilized, revealing a decrease in the number and strength of communications between NKT cells and other cells in sepsis (Figure 3G-H).
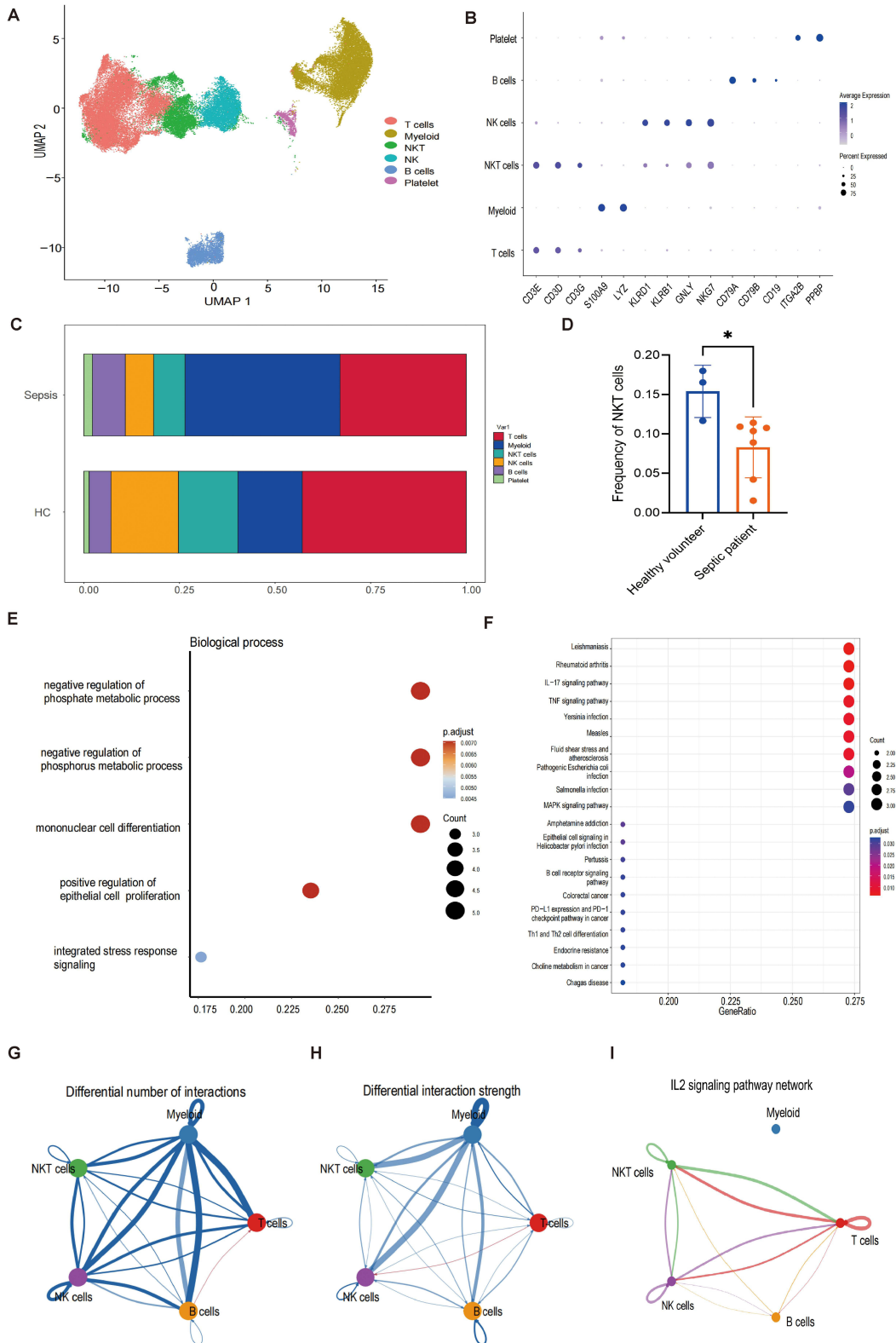
Figure 3 Cellular populations identified and function of NKT cells. (**A**) The UMAP plot displays the cellular composition. (**B**) Dotplot shows the marker gene expression in indicated cell types. (**C**) Bar plot indicates the frequencies of each cell type between HC group (n=3) and sepsis group (n=7). (**D**) The frequency of NKT cells in PBMC between HC group (n=3) and sepsis group (n=7). The Student's t–test was performed. *P<0.05. (**E**) Biological process of GO enrichment analysis. (**F**) KEGG enriched pathways of differentially expressed genes in NKT cells between HC and septic patients. (**G** and **H**) Diagrams display the differential number of interactions and differential interaction strength in cell clusters in sepsis compared to HC. (**I**) IL-2 mediating signaling pathway in sepsis.

Specifically, IL-2-mediated signaling pathway interactions between NKT cells, B cells, and T cells (Figure 3I). Ligand-receptor pair analysis showed reduced interactions from NKT cells to T cells (IL2-IL2RB+IL2RG) in sepsis compared to controls (Figure S2). This decreased interaction, particularly with adaptive immune cells, may contribute to the compromised immune response in sepsis.

The Distinctive FOS⁺NKT Cells Decreased in the Septic Patients

To comprehensively understand changes in immune infiltration by NKT cells in septic patients, we utilized unsupervised clustering and UMAP visualization, identifying six distinct NKT cell clusters (Figure 4A). Visualization of the top 15 expressed genes for each cluster revealed distinct gene modules associated with each subgroup (Figure 4B). These subgroups were classified as TRBV9⁺NKT cells, TRBV4-1⁺NKT cells, FOS⁺NKT cells, SDPR⁺NKT cells, KLRC1⁺NKT cells, and PTGDS⁺NKT cells, based on their heightened expression of specific genes (Figure 4C). Compared to healthy controls, FOS⁺NKT cells were decreased in septic patients (Figure 4D).

To understand the developmental trajectory of NKT cells, we performed a pseudo-time analysis using Monocle 2. FOS⁺NKT cells were predominantly found in later pseudo-time stages, as shown in the pseudo-time density plot (Figure 5A-B). Additionally, genetic changes within these cell clusters were examined throughout pseudo-time using a branched heatmap illustrating the dynamic expression of the top 50 significant genes within distinct cell fate branches (Figure 5C). In early pseudotime stages, genes associated with ribosome activity like *RPS18, RPLP1*, and *PRL39* were upregulated, indicating active ribosome biogenesis in NKT cells. As pseudo time progressed, there was a dynamic shift towards a functional profile, marked by elevated expression of key immune response factors including *GZMA, S100A4, IRF1, ITGB2*, and *CCL4*, all of which are recognized for their pivotal roles in the immune response to infection and inflammation. This shift underscores the potential central role of FOS⁺NKT cells in sepsis.

Dysfunction of FOS⁺NKT Cells in Sepsis

To gain insight into the function of genes in FOS⁺NKT cells during sepsis, we performed KEGG analysis, revealing enrichment in pathways such as the MAPK signaling pathway, IL-17 signaling pathway, TNF signaling pathway, B cell receptor signaling pathway, and T cell receptor signaling pathway (Figure 6A). This suggests the crucial role of FOS⁺NKT cells in activating the adaptive immune response. Additionally, transcription factor analysis showed significant enrichment of *FOS* in FOS⁺NKT cells (Figure 6B), indicating its involvement in gene regulation, differentiation, and responses to stress and inflammation.

The results of CellChat showed that the number of interactions and interaction strength between FOS⁺NKT cells and other immune cells were decreased in sepsis compared to healthy controls, except for its interaction strength with NK cells (Figure 6C-D). We then explored important ligand-receptor pairs sent from the FOS⁺NKT cells to other cell types. We found that ligand-receptor pair IL2-(IL2RB+IL2RG) and IL16-CD4 were decreased in sepsis from FOS⁺NKT cells to T cells and BTLA-TNFRSF14 and MIF-(CD74+CD44) ligand-receptor pair were decreased from FOS⁺NKT cells to B cells (Figure 6E-F). CCL3-CCR5 and IL7-(IL7R+IL2RG) increased from FOS⁺NKT cells to NK cells. The insufficient activation of T cells and B cells due to a reduction of function in NKT cells, especially the FOS⁺NKT cell subtype, and enhanced activation of inflammatory NK cells may be contributing factors to the progression of sepsis.

Discussion

Despite NKT cells' integral role in immune response, little is understood about how NKT cells may exert impact on sepsis. By utilizing MR analyses based on the analysis of GWAS data, a valid test for the presence of a causal relationship between NKT cells and sepsis can be provided. Our research highlights the particular and protective function of NKT cells in preventing sepsis-related death. To our knowledge, this is the first MR analysis to explore the causal relationship between NKT cell traits and sepsis. We then conducted a prospective and observational study to evaluate the percentage of NKT cells in septic patients, and we found that the percentage of NKT cells was significantly decreased in 28-day non-survivors, which plays a crucial supporting role in aiding clinical assessments of disease prognosis.

Subsequent analysis of immune cell frequencies via single-cell sequencing data revealed a decrease in NKT cells in sepsis. NKT cells are a type of lymphocyte that plays a unique role in the immune system. They are known for their
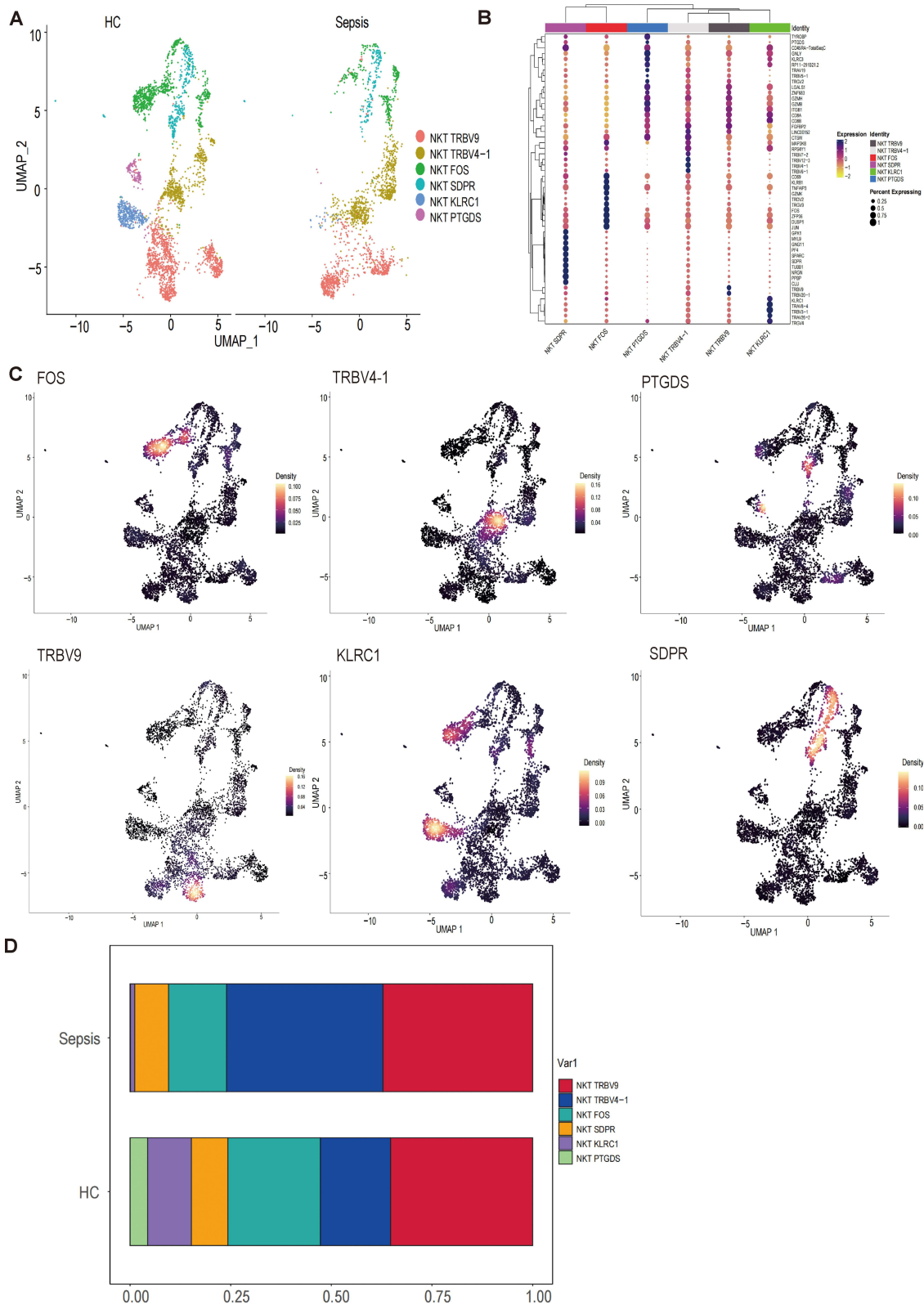
Figure 4 Distinctive FOS+NKT cells decreased in the septic patients. (**A**) The UMAP projection of NKT cells shows the formation of 6 clusters with the respective labels. (**B**) Top 15 genes expressed in different NKT subtypes. (**C**) Marker gene expression in NKT cell subtypes. (**D**) The average percentage of each cell type between HC and septic patients.
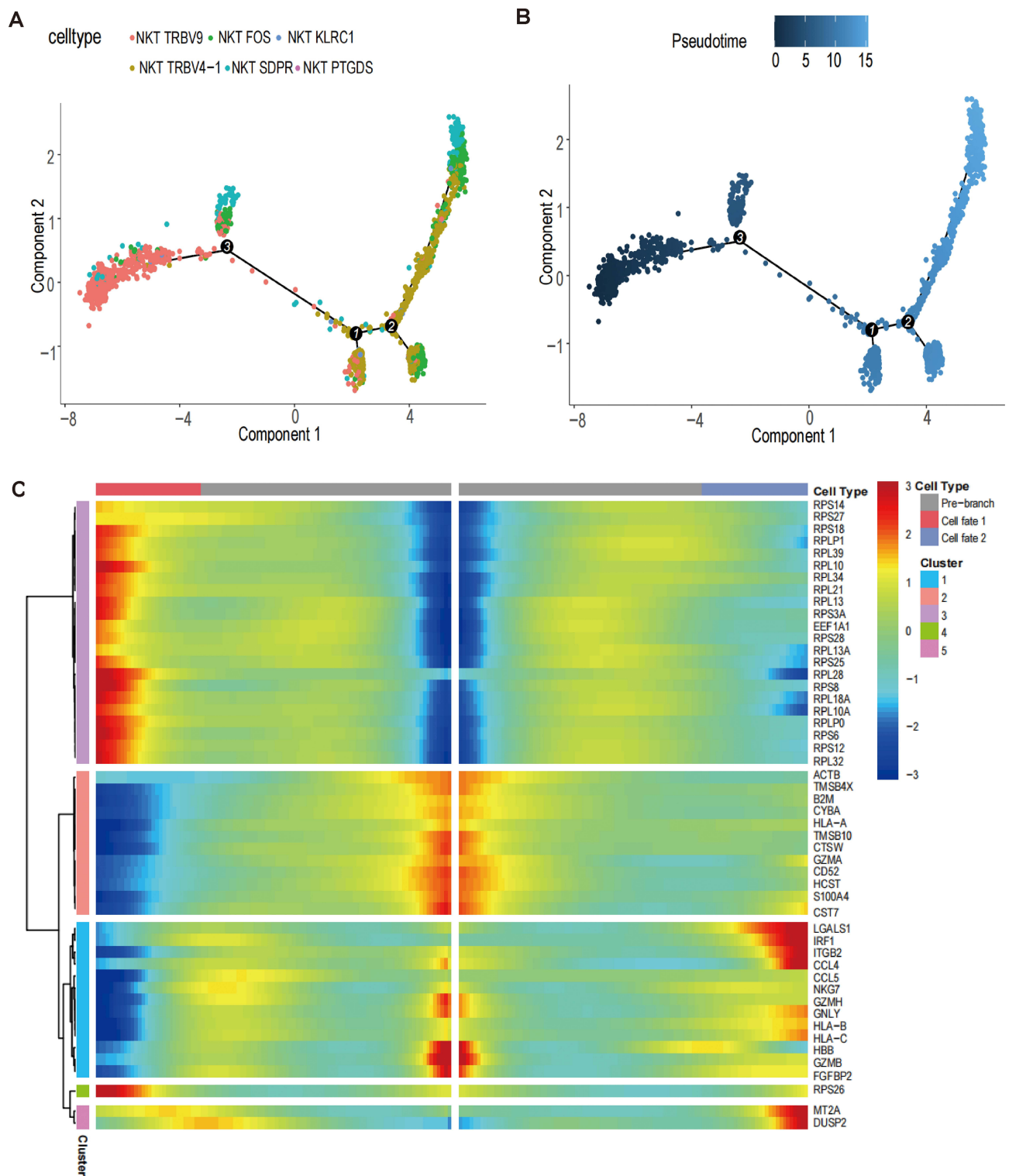
Figure 5 Pseudo-time series analysis of NKT cells. (**A** and **B**) Monocle2 is used to infer the developmental trajectory of NKT subsets. (**C**) The patterns of gene expression along with the pseudotime.

ability to bridge the gap between the innate immune system and the adaptive immune system, which creates specific immune responses tailored to specific pathogens. The reduction in NKT cells suggests potential dysregulation within immune cells during sepsis. Additionally, we found that the communications between NKT cells and other immune cells were decreased by Cellchat. The results showed that decreased interactions between NKT cells and other immune cells in

Figure 6 The cell-cell communication between FOS⁺NKT cells and immune cells. (**A**) Bar graph of KEGG enriched pathways of differentially expressed genes in FOS⁺NKT cells between HC and septic patients. (**B**) Heatmap shows NKT cell subtypes transcription factor activity. (**C** and **D**) Diagrams displaying the differential number of interactions and differential interaction strength between FOS⁺NKT cells and other immune cells in sepsis. (**E** and **F**) The communicated probability and related P values of the selected ligand-receptor interactions between FOS⁺NKT cells and other immune cells within the sepsis. Dot color reflects communication probabilities and dot size represents computed p-values. P < 0.05 refers to the significant ligand-receptor interaction.

sepsis were mainly caused by decreased IL2-(IL2RB+IL2RG) and MIF-(CD74+CD44) ligand-receptor pair. The decreased ability of NKT cells to activate other immune cells, especially adaptive immune cells, may lead to insufficient immune response and decreased anti-bacterial ability of the host in sepsis.

Through performing scRNA-seq on NKT cells, we identify a specific subtype of NKT cells in sepsis. FOS⁺NKT cells were found to decrease in septic patients, which were regulated by the transcription factor *FOS*. FOS⁺NKT cells are characterized by the elevated expression of crucial functional genes such as *GZMA, S100A4, IRF1, ITGB2*, and *CCL4*, all of which are recognized for their pivotal roles in the immune response to infection. Wang et al found that a type of circulating CD160^hiNKT cells was decreased in patients with CRKP infection, which is consistent with our finding, facilitating specific NKT subtype in host–immune response against infection.[12] Analysis of communication between FOS⁺NKT cells and other immune cells, we found that the number of interactions and interaction strength was decreased between FOS⁺NKT cells and adaptive immune cells, the decreased number and dysfunction of NKT cells, especially FOS⁺NKT cells, may lead to insufficient activation of T cells and B cells, promoting sepsis. According to the Cellchat, we found that interaction strength between FOS⁺NKT cells and NK cells was increased. It is reported that NKT cells can drive the production of IFN-γ by NK cells through the mTORC1 pathway, which impairs macrophage phagocytosis, and ultimately increases mortality.[24] Further investigation in FOS⁺NKT cells in sepsis is warranted.

There were several limitations existed in our study. Firstly, this study is limited to a single center, and its findings lack validation from external datasets. Secondly, the single-cell RNA sequencing study had a limited sample size. Thirdly, we did not account for the impact of infectious pathogens on NKT cells when including patients with sepsis. Finally, we have not yet clinically validated the expression of FOS⁺NKT cells in patients with sepsis, nor have we assessed the association between FOS⁺NKT cells and sepsis in the animal, future research will pay greater attention to this aspect.

Conclusions

In conclusion, we have demonstrated that NKT cells may provide feasible protection for patients with sepsis, particularly those with severe outcomes. Additionally, we identified a specific FOS⁺NKT subset that may contribute to sepsis due to its decrease and insufficient ability to activate the immune response.

Abbreviations

NKT, Natural killer T; MR, Mendelian randomization; PBMC, Peripheral blood mononuclear cell; IFN-γ, Interferon -γ; IL-4, Interleukin-4; CLP, Cecal ligation and puncture; RCT, Randomized controlled trial; CPKP, Carbapenem-resistant Klebsiella pneumonia; GWAS, Genome-wide association study; SNP, Single-nucleotide polymorphism; IV, Instrumental variable; LD, Linkage disequilibrium; IVW, Inverse variance weighting; scRNA-seq, Single-cell RNA sequencing; GEO, Gene Expression Omnibus; HC, Healthy control; PCA, Principal component analysis; UMAP: Uniform manifold approximation and projection; DEG, Differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TNF, Tumor necrosis factor; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.

Data Sharing Statement

The GWAS summary statistics for each immune trait are publicly available in the GWAS catalog (accession number from GCST90002084). The GWAS summary statistics for sepsis can be accessed through the IEU Open GWAS website with identifier ieu-b-5086 (for 28-day mortality).

The scRNA-seq data of septic patients and health controls used in this study are publicly available from the GEO database under accession numbers GSE151263 and GSE157007. All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics Approval and Consent to Participate

The study was approved by the research ethics committee of Zhongda Hospital (Southeast University, Nanjing, China, approval ID: 2020ZDSYLL308-P01) and was in full compliance with the Declaration of Helsinki. Informed consent was obtained from each patient or their legal representative before enrollment in the study.

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Disclosure

The authors declare no conflicts of interest in this work.

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