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ORIGINAL RESEARCH

Dysregulated Immune Responses in Sepsis: Insights From Treg-Related Gene Expression

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Introduction: Sepsis, a life-threatening dysregulated immune response to infection, has a high global mortality rate. Tregs play dual roles in sepsis pathogenesis, with their expansion linked to immunosuppression. This study explores Treg dynamics and the novel role of CD82 in sepsis.

Methods: Peripheral blood from sepsis patients was analyzed using scRNA-seq. Machine learning (SVM, LASSO, random forest) integrated scRNA-seq data with three GEO datasets (n=380) to identify biomarkers. CD82 expression in Tregs was validated via flow cytometry and RT-qPCR in CLP mouse model. Anti-CD25 antibody depleted Tregs in mice.

Results: The scRNA-seq revealed neutrophil expansion and T/NK cell reduction in sepsis. Tregs were enriched and exhibited CD82 upregulation. A seven-gene diagnostic signature (CD82, CD52, EVI2B, IL32, RCAN3, AQP3, NAP1L1) achieved high accuracy (AUCs up to 99.9%). Treg-depleted CLP mice showed reduced CD82 expression, elevated IL-6 and neutrophils, and worsened inflammation, implicating CD82 in immune modulation.

Discussion: CD82 may mediate Treg hyperactivation during sepsis, balancing the immune response and suppression. The gene signature shows diagnostic potential, but CD82's mechanistic role needs further investigation. Therapeutic targeting of CD82 could improve sepsis management.

Keywords: logistic regression, machine learning method, single-cell analysis, Treg, sepsis

Introductions

Sepsis is a leading cause of death among hospitalized patients worldwide. The disease progresses rapidly and has a high mortality rate and disease burden.[1] Sepsis is a life-threatening condition caused by the dysregulation of the body's inflammatory response to infection, leading to the excessive release of inflammatory mediators known as systemic inflammatory response syndrome (SIRS).[2–4] In patients with systemic inflammation and sepsis, platelet activation is commonly present, and there is an increase in immature neutrophil count (left shift), which is associated with clinical syndromes related to sepsis.[5–7] In addition to the immune cells being extensively researched, septic patients also experience notable alterations in lymphocytes and other cell types. In sepsis, there is an increase in the proportion of Treg cells due to enhanced resistance of Treg cells to lymphocyte apoptosis. This is a shared characteristic observed in humans and mice.[8] However, the immune dysfunction frequently seen in the later stages of sepsis or acute respiratory distress syndrome (ARDS) may be partly attributed to the suppressive role of Treg cells.[9] These research reports remind us that the immune response to sepsis involves the participation of multiple immune cells, prompting us to focus on the

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specific role of Treg cells in sepsis. Treg cells are crucial in maintaining tolerance, preventing inflammatory diseases, regulating immunity against infections, restraining tumor and anti-autoimmune responses, among other functions.

Treg cells are CD4+ T cells that suppress the immune system, maintaining immune tolerance and preventing autoimmune diseases.[10] Activation of Treg cells can be spontaneous (natural Treg cells) or induced (induced Treg cells). They recognize self or non-self antigens by interacting with antigen-presenting cells (APCs) and are activated in response to appropriate stimuli.[11] Repressive cytokines like IL-10 and TGF-β are secreted by Treg cells to hinder the activation and growth of autoreactive T cells, thus averting any potential harm to the body's tissues.[12] In chronic infections, such as HIV or tuberculosis, Treg cells may help limit tissue damage caused by pathogens, but at the same time may also limit the response of pathogen-specific T cells, affecting the clearance of pathogens.[13,14] Treg cells tend to undergo proliferation in the microenvironment surrounding tumours and assist in enabling tumour cells to evade detection by the immune system.[15] NK cells and CTLs' anti-tumor activity is suppressed by regulatory T cells, leading to the promotion of cancer growth, recurrence, and metastasis.[16]

Existing studies on the role of Treg cells in sepsis have shown that the number of Treg cells in the peripheral circulation of patients with sepsis increases and their regulatory function is enhanced, which is closely related to the severity of the disease.[17] In the early stage of sepsis, Treg cell function is inhibited; while in the immunosuppressive stage, Treg cells exhibit strong resistance to apoptosis, leading to an increased proportion and enhanced regulatory function.[18–20] The proportion of Treg cells in peripheral blood was negatively correlated with a simplified acute physiological score II of sepsis and blood lactate level, indicating that the change of Treg cell proportion could reflect the severity of sepsis.[21] In addition, increased expression of Foxp3, a key transcription factor in Treg cells, is associated with poor prognosis in patients with sepsis.[22]

The early injection of exogenous regulatory T cells significantly improves the survival rate of septic mice, indicating that intervention with regulatory T cells at different stages may be an effective strategy to improve the immune status in sepsis.[8] In conclusion, Treg cells are crucial in sepsis' pathogenesis, progression, and prognosis. Further investigation into the underlying mechanisms of Treg cells in sepsis is anticipated to provide novel insights and approaches for diagnosing and treating this condition. This study aimed to investigate the immune response in septic mice and its potential implications. The researchers found that there was an increased proportion of peripheral blood Treg cells in septic mice compared to healthy controls. To further understand the role of these cells, they were isolated and analyzed using flow cytometry. Interestingly, the analysis revealed abnormal upregulation of CD82 expression within these Treg cells. CD82 is a protein involved in cell adhesion and migration, but its specific role in regulating immune responses is not yet fully understood.[23] CD82 seems to recruit T cell polarity-regulating molecules such as Scribble via the PDZ domain-binding motif located in its C-terminus and mediate the T cell polarization.[24] No studies on CD82 and Treg have been reported. However, this study found that in the case of sepsis, Treg cells would upregulate the expression of the CD82 molecule, suggesting that CD82 may play a significant role in modulating the function of Treg cells during sepsis.

Method

Animals and CLP Model

All animals used in this experiment were 8–10 weeks old C57BL/6 mice (half male and half female), with a basic weight of 20–25g, purchased from Shulaibao (Wuhan, China) Biotechnology Co., Ltd., and maintained in an animal center, Huazhong University of Science and Technology. Animals are raised in a specific pathogen-free (SPF) environment, with free access to food and water, following a strict 12-hour light-dark cycle, and temperature controlled at 22–24°C with humidity maintained at 60–65%. According to the general guidelines, a cecal ligation and puncture (CLP) surgical operation was performed to establish a sepsis model in vivo.[25] All animal experiments met the requirements of the National Institutes of Health (NIH) guidelines and were approved by the Laboratory Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The animal ethics committee approval number is 3596.

Single-Cell RNA Sequencing Analysis

The analysis of sc-RNA seq was conducted using Seurat (4.4.0), an R package designed for single-cell analysis.[26] We re-analyzed published single-cell RNA-seq data from ICU patients and healthy donors,[28] with clinical metadata and processed matrices available at Zenodo (https://doi.org/10.5281/zenodo.7723202).[28] After removing the duplicate detection samples, there were 15 healthy controls and 18 sepsis patients in the SeuratObject. The RunHarmony function was applied to correct batch effects among datasets and samples, with the parameters theta and lambda set as one for these two variables. Cell clustering was performed via Seurat's SNN graph (resolution=0.6), followed by UMAP visualization (dims=1:40, reduction="harmony").Notably, we removed clusters expressing signatures of other major cell types and the above steps were repeated to ensure that we have filtered out these cell doublets. For any unspecified parameter, we used the default settings. Major clusters were annotated using canonical markers. The second round clustering procedure was the same as the first-round clustering, both of which started from the logarithm-transformed matrix, and then identified the top 3000 highly variable genes, calculated PCA matrix and corrected batch effects using Harmony. The batch effect correction parameters used were as follows: T/NK_cells: batch_ID = "orig.ident". The resolution parameter used was as follows: 0.7. To accurately define the cell types and cellular states, differentially expressed genes were identified for each cell cluster using the FindAllMarkers function in Seurat. Gene clustering results are shown in Tables S1 and S2.

Microarray Data Processing

We used the GEO (http://www.ncbi.nlm.nih.gov/geo) database to filter the microarray data about sepsis and normal samples, including GSE54514,[29] GSE65682,[30] and GSE95233.[27] To ensure the data quality, data filtering, background adjustment, log2 transformation, and normalization preprocessing were carried out on the three original datasets. In addition, the "Combat" algorithm was also used to correct the batch effect among these three datasets. The merged dataset contains 380 samples, including 280 sepsis samples and 100 normal samples.

Machine Learning

The parameter alpha of LASSO regression is 1 and cross-validation is used to select the optimal penalty parameter (lambda.min = 0.001644006). The mSVM-REF function (https://github.com/johncolby/SVM-RFE) is used for identification and screening of key genes (k = 10, halve.above = 100, and other default parameters are used). The randomForest function of the randomForest package is used for screening key genes in random forest (default parameters are used).

Predictive Model for Sepsis About Treg Cell

The merged dataset was randomly divided into a training set (70%, N = 266) and a test set (30%, N = 114). The diagnostic nomogram of sepsis was constructed by fitting the characteristic genes to the binary logistic regression model with the training set. The predictive performance of the nomogram was evaluated using calibration curves, decision curve analysis (DCA), and the area under the receiver operating characteristic curve (AUC) value. To further estimate the discriminatory ability of the characteristic genes for sepsis patients and controls, we used three validation datasets: GSE54514, GSE65682, and GSE95233, and drew the AUC through the "pROC(1.18.5)" package.

Tissue Distribution Preference of Cell Subsets

To evaluate the tissue distribution preferences of cell subsets, we computed the ratio of observed to expected cell counts (Ro/e) for each annotated cluster across various tissues. The expected cell counts for clusters in each tissue were determined using the chi-square test. A Ro/e value exceeding one suggests that a cluster is enriched in a particular tissue.

Quantitative Reverse-Transcription–PCR (RT-qPCR)

Total RNA was extracted by the TRIZOL method and reverse-transcribed using a reverse transcription reagent (Vazyme). For the quantitative real-time PCR (RT-qPCR),[31] reaction mixtures were prepared containing cDNA, forward and reverse primers, and SYBR™ Green PCR Master Mix (Bio-Rad, USA). The reactions were performed in a QuantStudio™ 3

Real-Time PCR System (Thermo Fisher Scientific, USA). CD82 primer sequences were: forward primer 5'-CAGCCTCCCGGTTCAATCAA, reverse primer 5'-CAGTTGGAGGCCTAAGGGTG.

Flow Cytometry

We performed flow cytometry on mice according to the method recommended in the guideline.[32] Mouse periphery blood underwent a process of eliminating red blood cells using Red Blood Cell Lysing Buffer (Sevier, Wuhan). After Fc-receptor blocking, cells were stained for surface markers in FACS buffer. Neutrophils were evaluated using CD11b+ and Ly6G+ antibodies through flow cytometry. Identification and sorting of CD4+ CD25+ Foxp3+ Treg cells in mouse peripheral blood; detection of CD82 cell-surface expression on Treg cells using the antibody against the extracellular domain of the CD82. All antibodies are commercially available from BioLegend.

Treg Depletion

Although there is currently no definitive approach to completely exhaust Tregs, even a partial eradication can alter their regulatory function.[33,34] Depletion of Tregs was performed by intraperitoneal injection of 500 μg anti-CD25 Ab (PC61) (BioXcell).[35] In the previous experiments, we observed through flow cytometry that the proportion of Treg cells decreased significantly 3 days after anti-CD25 treatment. The CLP and the control group underwent Treg depletion three days before (n=6 per group).

ELISA Assays

Enzyme-linked immunosorbent assay (ELISA) is an immunoassay technique that combines the antigen-antibody immune reaction and the highly efficient catalytic effect of enzymes.[36] Mouse IL-6 ELISA Kit (eBioscience, USA) was used to measure the expression levels IL-6 in plasma from CLP mice and the healthy controls according to the manufacturer's instructions (n=6 per group).

Statistical Analysis

The data were presented as mean ± standard deviation (SD) and analyzed using GraphPad Prism 10 software (GraphPad Software, USA). Student's *t*-test was used to detect the significance of differences between samples. The programming language used for the main analysis was R version 3.5.3.***statistical difference at $p \leq 0.001$, **statistical difference at $p \leq 0.01$ and *statistical difference at $p \leq 0.05$.

Ethical Statement

The present study did not require ethical approval because it is based on de-identified publicly available data. According to the item 1 of Article 18 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China. This study uses legally public data or biological samples that cannot be identified as specific subjects to carry out research. Moreover, the research activities did not cause risks to the privacy and rights of the subjects, which met the ethical exemption conditions.

Results

Single-Cell Analysis of Sepsis Blood Samples

Based on a peripheral blood whole-blood single-cell study related to sepsis,[28] removing batch effects using Harmony, we performed initial cell population annotation and identified six cell clusters (mature neutrophils, immature neutrophils, T/NK cells, platelets, monocytes) (Figure 1A). The characteristic annotated genes are shown in Figure 1B. Neutrophil cell cluster is significantly different and more prevalent in sepsis. We established a CLP mouse model and observed a significant increase in the proportion of neutrophils compared to the sham surgery group, indicating that an animal model has been successfully established for subsequent experiments (Figure 1D). Furthermore, we also observed significant differences in T/NK cells during the disease course with decreased ratios of this cell cluster in septic patients (Figure 1C). The Ro/e scoring results do not indicate enrichment of T/NK cells in sepsis group (Figure 1E). Furthermore,

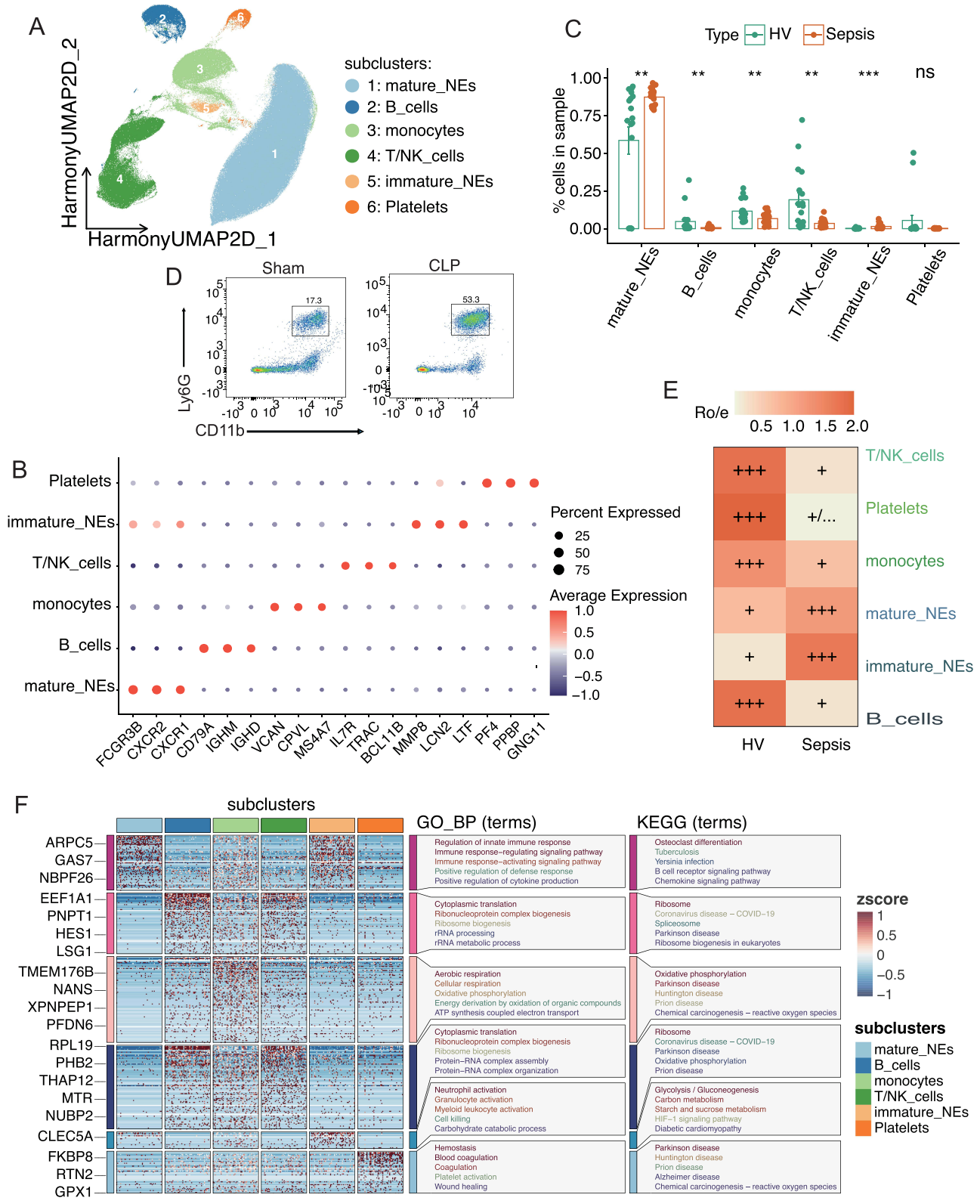

Figure 1 (**A**) UMAP plots showing 6 types of immune cells, color-coded according to different clusters. (**B**) Dot plot showing the expression of selected marker genes in immune cell types. (**C**) *T* test shows the cell proportions of different cell types of the HV group (n=15) and the sepsis group (n=18). (**D**) Flow cytometry revealed that circulating neutrophil numbers were significantly increased in the CLP group. (**E**) Heatmap shows the enrichment of immune cells in the HV and Sepsis groups. +++, Ro/e > 1; ++, 0.8 < Ro/e < 1; +, 0.2 < Ro/e < 0.8; +/…, 0 < Ro/e < 0.2; Ro/e > 1 was considered as enriched in the group. (**F**) The complexheatmap displays the top 20 genes exhibiting the highest degree of differential expression. Additionally, it presents the enrichment results for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses corresponding to these highly ranked genes. ***P < 0.001; **P < 0.01.

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we conducted the functional enrichment analysis of these DEGs using GO and KEGG databases and list the up 20 genes (Figure 1F).

Subclassification and Functional Analysis of T/NK Cells

The UMAP plot illustrates the clustering and cell type annotation of T/NK cells (Figure 2A). Based on differential genes expression, T/NK cell subgroups was further subclassified as 8 clusters on the UMAP plot, namely NK_GNLY, NKT_NKG7, CD8T_CX3CR1, CD8T_GZMK, CD4T_FOXP3, CD4T_GPR183, CD4T_FOS, and naiveT. By utilizing genes such as TCF7, CCR7, SELL, CD4, FOS, JUND, RPS18, GPR183, LTB, IL6R, IL7R, FOXP3, CD8A, C D8B, GZMK, CX3CR1, SH3BGRL3, CD52, GZMH NKG7, CCL5, GNLY, FGFBP2, FCGR3A (Figure 2C). The proportion of Treg cells was found to be increased in the sepsis, as indicated by the Ro/e ratio suggesting their enrichment in the sepsis group (Figure 2B–D). We performed functional enrichment analysis on different subtypes of T/NK cells and found that Treg cells were involved in lymphocyte differentiation and cell adhesion molecules (Figure 2E). To further investigate the role of Treg cells in sepsis, we selected 49 differential genes for subsequent analysis.

Machine Learning-Based Gene Selection for Sepsis Diagnosis

Subsequently, we analyzed three sepsis datasets in the GEO, which included a total of 380 samples. After removing batches, we merged them into a large dataset (sfigure 1). By integrating single-cell analysis data and identifying the intersection of bulk analysis genes, we derived 37 genes set. Machine Learning techniques were then applied to determine the best-fitting variables from these gene sets. The Support Vector Machine (SVM) model exhibited the highest 5-fold cross-validation accuracy and the lowest error rate using 21 genes (Figure 3A and B). LASSO regression, based on the regularization parameter (λ), identified 19 key features in the model (Figure 3C and D). Using the R package "randomForest", we constructed a random forest model, identifying the top 10 most important genes (Figure 3E and F). Finally, we further combined three machine learning algorithms to narrow down the gene range and ultimately obtained seven genes is represented in each intersection of the Venn diagram: CD82, CD52, EVI2B, IL32, RCAN3, AQP3, NAP1L1 (Figure 3G and H).

Validation of the Sepsis Diagnostic Model

In the bulk analysis, a total of 380 patients were included and the measured values of 7 genes were retained. The logistic regression model fitted by lrm() was utilized for collinearity diagnosis, revealing that all variance inflation factor (VIF) values were below 5 and no removal was necessary which resulted in an increased probability of sepsis diagnosis (Figure 4A). To randomly divide the dataset into training set and test sets, a ratio of 0.7 was applied to the 380 cases of data. Decision curve analysis (DCA) was then performed using the DCA R package, plotting net benefit rate against high-risk threshold on the abscissa axis (Figure 4B and C). Additionally, ROC curve analysis demonstrated that these genes exhibited diagnostic performance for sepsis in both the test set and train set (Figure 4D and E). Finally, this diagnostic model's effectiveness was verified across three datasets with respective AUCs of (99.9%, 80.4%, 99%) (Figure 4F–H).

In Vivo Role of Treg Cells and CD82 in Sepsis

We observed in a mouse model of sepsis induced by CLP that the proportion of Treg (foxp3+CD25+) cells in peripheral blood gradually increased in the first three days as shown by flow cytometry analysis (Figure 5A–I). Additionally, we found that CD82 molecule expression was upregulated on Treg cells on the second and third day (Figure 5J–M). RT-qPCR revealed an upregulation of CD82 expression in peripheral blood after CLP surgery, indicating the involvement of CD82 molecule in the regulation of sepsis pathogenesis (Figure 5N). Subsequently, we depleted Treg cells in vivo by intraperitoneal injection of anti-CD25 antibody. Although complete depletion was not achieved, we were able to reduce the proportion of Treg cells. The flow cytometry analysis conducted on the third and fifth day following intraperitoneal injection revealed persistent low levels of Treg cells (sfigure 2). We then modeled CLP-induced sepsis in mice with Treg-depleted and observed a relatively lower expression of CD82 compared to WT mice (Figure 6A). Furthermore, measurement of neutrophil proportions and plasma IL-6 levels reflected inflammatory status. Flow cytometry showed

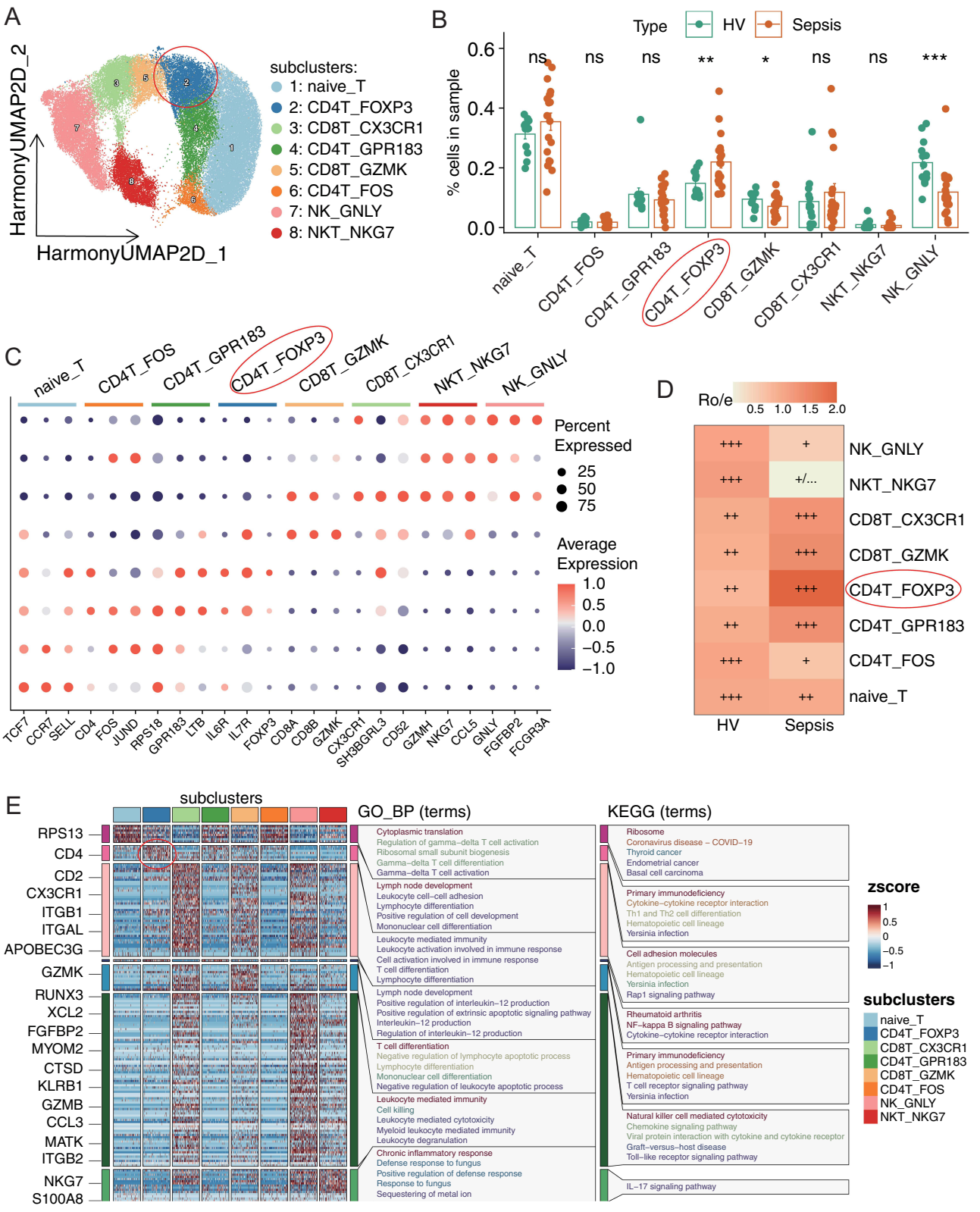

Figure 2 (**A**) UMAP clustering of T/NK cell subsets, population 2 is an identified subset of Treg (CD4T_FOXP3) cells. The red circle outlines the Treg cells. (**B**) Student't test shows the cell proportions of different subset of the HV group (n=15) and the sepsis group (n=18). (**C**) Dot plot showing the expression of selected marker genes in subpopulations. (**D**) Heatmap shows the enrichment of subpopulations in the HV and Sepsis groups. +++, Ro/e > 1; ++, 0.8 < Ro/e < 1; +, 0.2 < Ro/e < 0.8; +/…, 0 < Ro/e < 0.2; Ro/e > 1 was considered as enriched in the group. (**E**) The complexheatmap displays the top 20 genes of subpopulations exhibiting the highest degree of differential expression. Additionally, it presents the enrichment results for GO and KEGG analyses corresponding to these highly ranked genes. ***P < 0.001; **P < 0.01; *P < 0.05.

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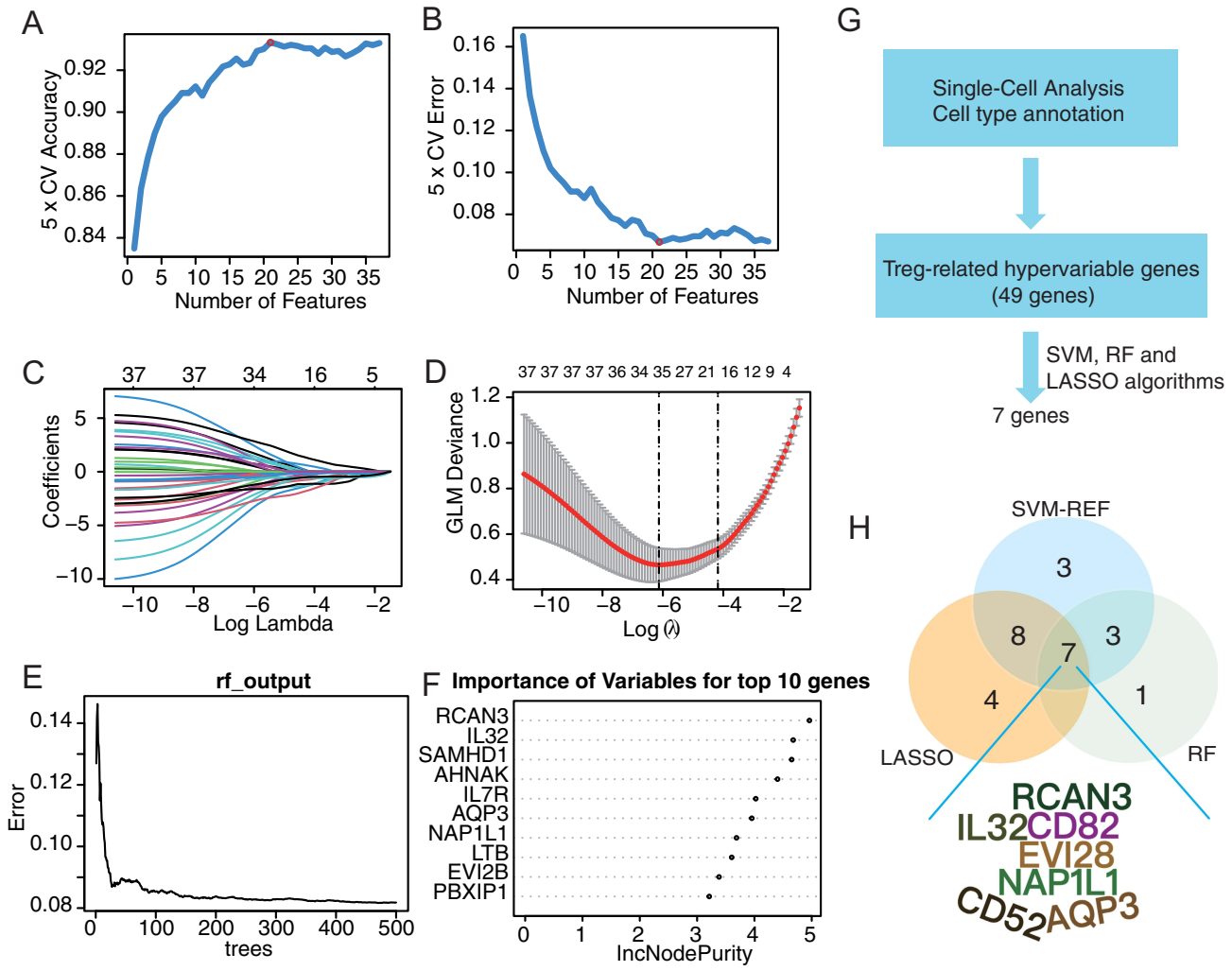

Figure 3 (**A** and **B**) Multiclass support vector machine (SVM) classifiers were trained using 5-fold cross validation. (**C**) Selection of Variables using Least Absolute Shrinkage and Selection Operator (LASSO) regression, LASSO coefficient of 37 variables in the model. (**D**) The optimal penalty coefficient in the LASSO regression was identified with the minimum criterion and selecting 21 optimal variables. (**E**) The influence of the number of decision trees on the error rate. (**F**) The importance of the top 10 genes was identified based on the algorithm requirements of the random forest. (**G**) Flow chart of the process of selecting genes. (**H**) A Venn diagram to show the intersection of optimum features for the three machine learning models.

that Treg-depleted mice neutrophils increased significantly after CLP compared with the WT (sfigure 3). On the first three days after CLP surgery, higher neutrophil proportions and IL-6 levels were observed in Treg-depleted mice compared to WT mice, suggesting a more severe inflammatory state (Figure 6B and C).

Discussion

Bioinformatic analysis of sc-RNA seq plays an important role in medical research. This study analyzed sc-RNA seq data from a large cohort of sepsis patients and combined it with microarray data to validate the genes associated with Treg cells that may be involved in the pathogenesis of sepsis. Ultimately, seven upregulated genes were identified as potentially participating in the pathogenesis of sepsis through Treg involvement. EVI2B is essential for granulocyte differentiation and function by controlling the cell cycle progression and survival of hematopoietic progenitor cells.[37] IL32 is a cytokine that may play a role in innate and adaptive immune responses, inducing various cytokines such as TNF-α and IL8.[38] CD82 is a component of specialized membrane microdomains called tetraspanin-enriched microdomains (TERMs), serving as a platform for receptor clustering and signal transduction.[39] CD82 inhibits the migration of neutrophils and macrophages to tissues, demonstrating its important role in suppressing phagocyte infiltration and mediating their differentiation.[40] Furthermore, studies have demonstrated that CD82 acts as a potent suppressor of

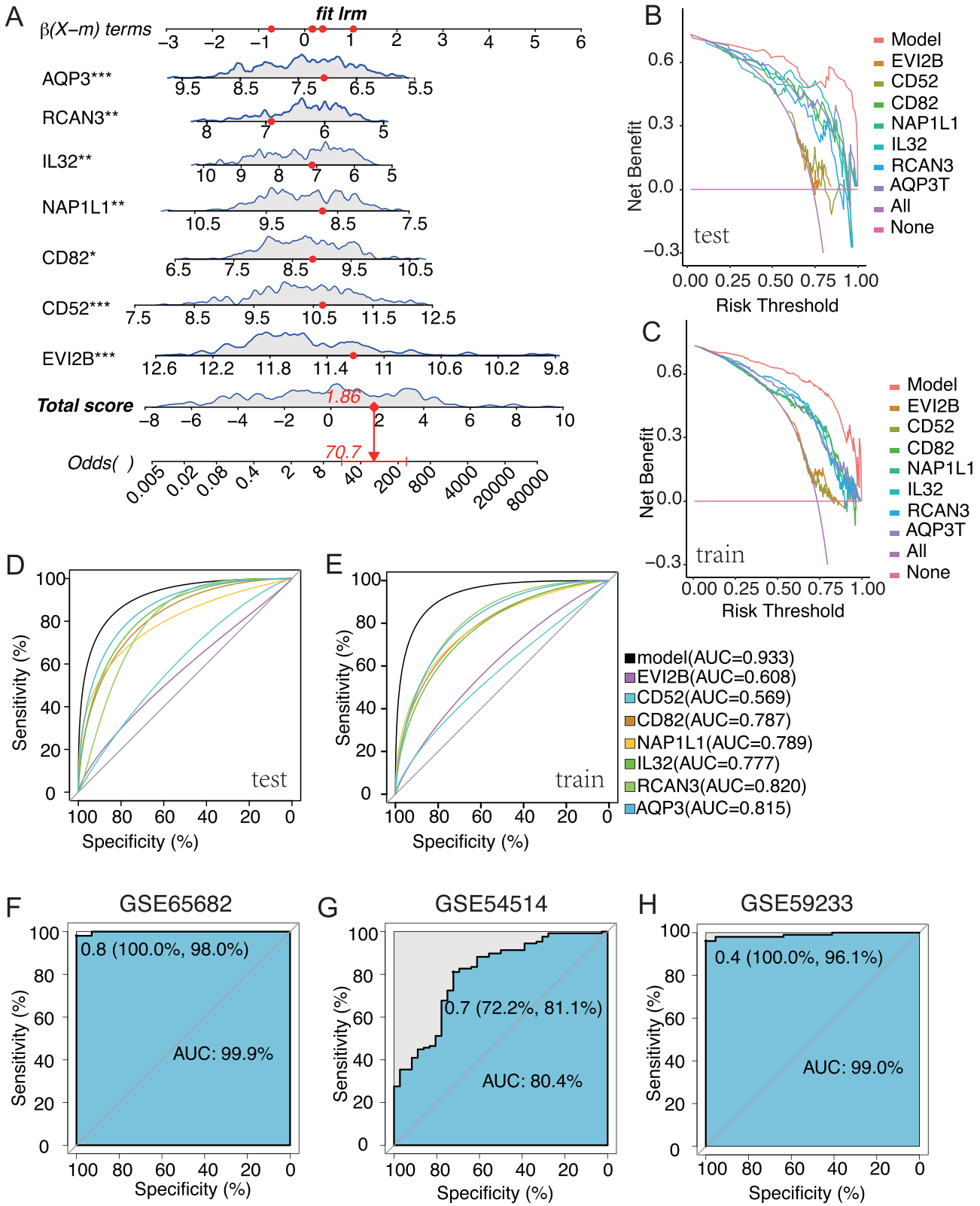

Figure 4 Establishment and performance of the logistic regression model. (**A**) Nomogram to predict the occurrence of Sepsis. (**B** and **C**) Show that decision curve analysis (DCA) was conducted for the prediction model based on the logistic regression model in the train and test datasets. (**D** and **E**) ROC curve with training datasets and test datasets using the logistic regression model with the 7 variables. (**F–H**) The ROC curve of 7 essential gene combinations is based on the logistic regression model. The different coloured lines represent different genes. ***P < 0.001; **P < 0.01; *P < 0.05.

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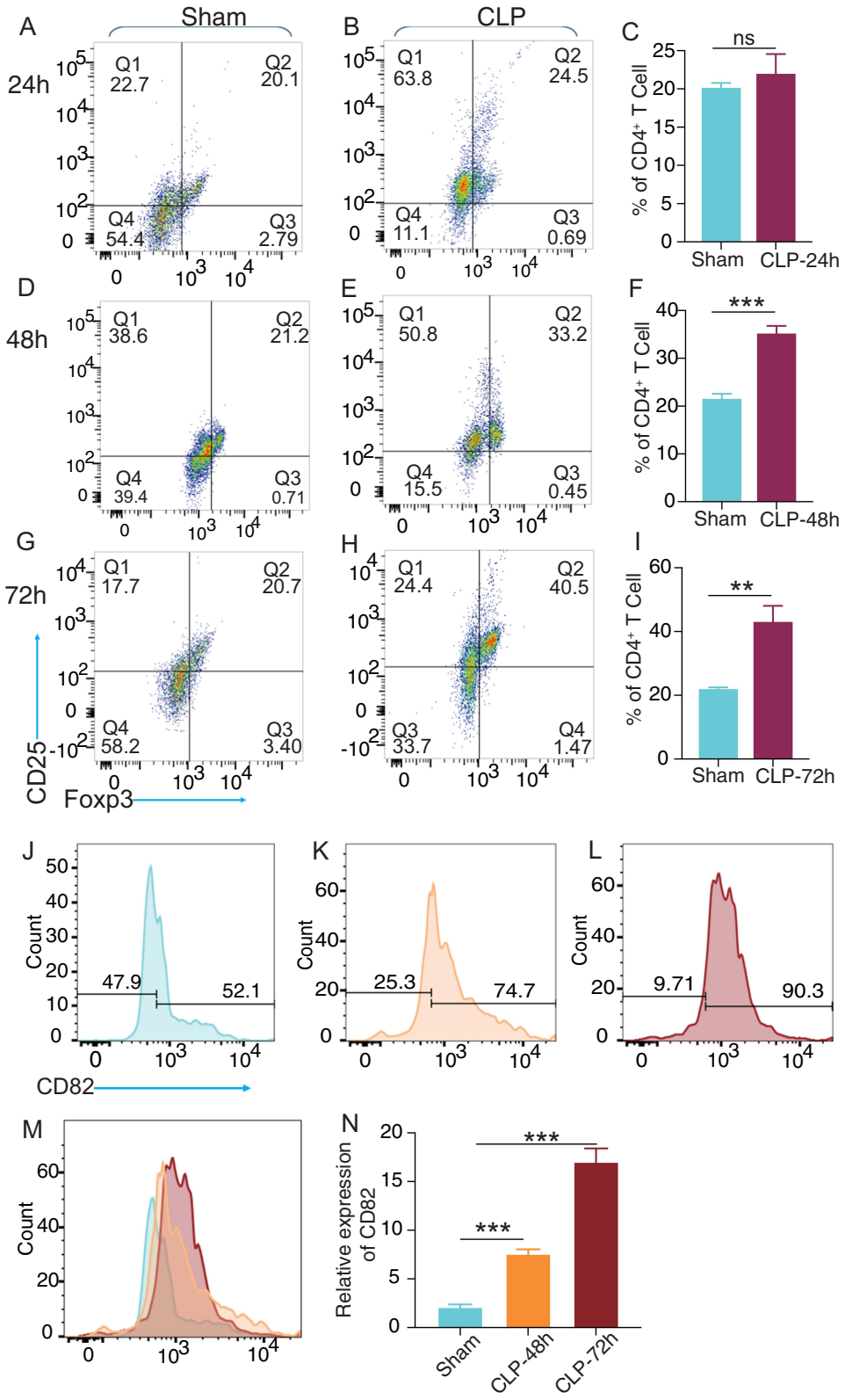

Figure 5 Flow cytometry detects Treg (CD4+CD25+Foxp3+) population in peripheral blood. (**A–I**) Mice were sacrificed at 24-, 48-, and 72-hours post-CLP for assessment of Treg in blood using flow cytometry compared with the Sham (n=6 per group). (**J–M**) The level of CD82 expression in Tregs was determined by flow cytometry (the Sham vs CLP48h group and CL72h group, n=6 per group). (**N**) qRT-PCR examined mRNA expression of CD82 in the peripheral blood of mice. ***P < 0.001; **P < 0.01.

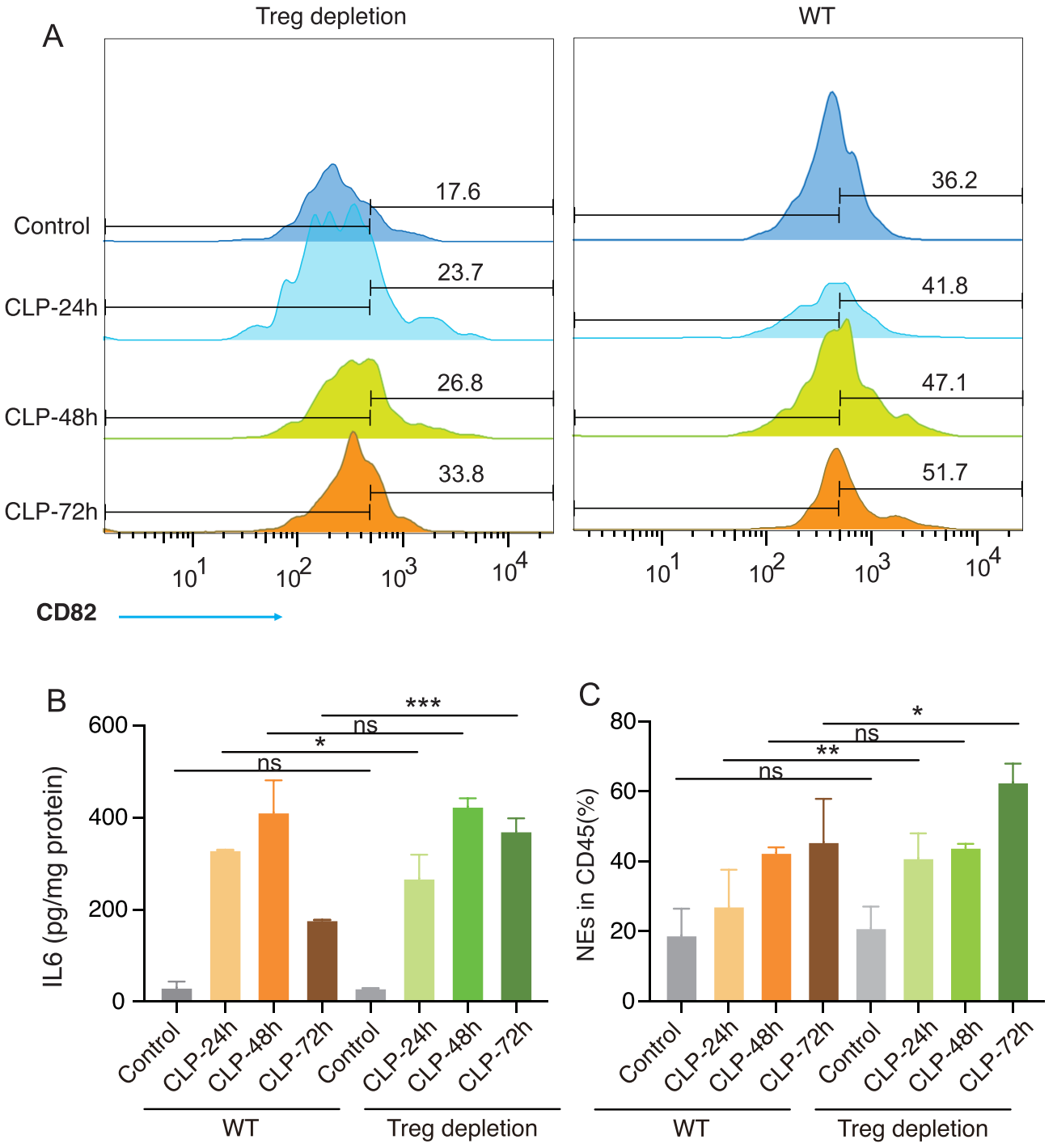

Figure 6 (**A** and **B**) The expression level of CD82 on Treg cells of Treg depleted and WT mice at different periods was detected by flow cytometry (n=6 per group). (**B**) The plasma levels of IL-6 were detected by the ELISA method (n=6 per group). (**C**) Flow cytometry analysis of neutrophil (CD11b + Ly6G +) population in blood (n=6 per group). ***P < 0.001; **P < 0.01; *P < 0.05.

tumour metastasis while also governing various cellular processes such as regulating the proliferation and chemotherapy resistance of AML cells via activation of the Wnt/β-catenin pathway.[41] Evolutionary analysis of CD82-like molecules in jawless vertebrates suggests their involvement in the immune response process, with upregulated expression observed in immune-related tissues following antigen stimulation.[42] Notably, CD82 confers protection against DSS induced colitis in mice by inhibiting BRCC3-mediated K63-specific NLRP3 deubiquitination through direct interaction with BRCC3 thereby attenuating NLRP3 inflammasome activation under inflammatory conditions.[43] These findings demonstrate

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that CD82 is indispensable for maintaining the delicate balance between tolerance and inflammation, modulating immune responses in homeostasis and pathologies. Physiological indices such as SI, MSI, and ASI have also shown predictive value for mortality in sepsis patients and may complement molecular approaches like CD82-based risk assessment.[44]

CD82 acts as a co-stimulatory molecule for T cell activation and may be involved in the activation of Treg cells. In septic mice, elevated CD82 levels potentially mediate the hyperactivation of Treg cells, which could contribute to sepsis-associated immunosuppression. However, further molecular biology research is required to elucidate the specific mechanism by which CD82 expressed in Treg cells regulates inflammation and immune cell differentiation in septic diseases. The drawback is that no mature CD82 antibodies are currently available to counteract the biological effects of CD82 molecules in vivo. If there are subsequent inhibitors targeting the CD82 molecule, conducting large-scale biological experiments or clinical cohort studies will better explain the role of CD82 in sepsis.

Conclusion

This study employed sc-RNA seq to analyse peripheral blood immune cells in sepsis, identifying significant alterations in neutrophil and T/NK cell populations. T/NK cell subsets, including regulatory T cells (Tregs), exhibited dynamic changes, with Tregs enriched in sepsis and associated with lymphocyte differentiation and cell adhesion pathways. Integration of three GEO sepsis datasets (n=380) and machine learning (SVM, LASSO, random forest) identified seven key diagnostic genes (CD82, CD52, EVI2B, IL32, RCAN3, AQP3, NAP1L1). A logistic regression model demonstrated strong diagnostic performance (AUCs: 99.9%, 80.4%, 99.0% across datasets). In this study, we found that the expression of the CD82 molecule was upregulated in peripheral blood Treg cells during sepsis. Flow cytometry analysis also revealed an upregulation of CD82 expression in Treg from CLP mice. Subsequently, we further investigated the role of CD82 in Treg by depleting Treg in mice. In Treg-depleted mice, higher levels of inflammation and an elevated ratio of peripheral blood neutrophils are observed, indicating that the early action of Treg is to reduce inflammation. This study explores the role of the CD82 molecule in Treg and reveals a decrease in its expression level upon Treg cell depletion, suggesting that CD82 may play a protective role in sepsis by regulating the function of Treg cells during sepsis.

Data Sharing Statement

The data used to support the findings of this study are included within the article.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

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