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Immune infiltration analysis reveals immune cell signatures in human and mouse model of primary Sjögren's syndrome in salivary gland tissue

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Abstract

Background: The mouse model is the basis for primary Sjögren's syndrome research, but the depth of comparison between mice and humans in salivary gland immune cells is limited.

Methods: Firstly, the gene expression profile of salivary glands of normal human and pSS patients was downloaded from the Gene Expression Omnibus database. The proportion of infiltrating immune cell subsets was then assessed by cell type identification by estimating relative subsets of RNA transcripts (CIBERSORT). The experimental Sjögren's syndrome (ESS) model mice was successfully constructed using the salivary gland (SG) protein of the salivary gland, and the successful mice were screened by measuring the amount of saliva and histopathological score. Based on mouse salivary gland tissue RNA-Seq data, the seq-ImmuCC model was used to quantitatively analyze the composition ratio of 10 immune cells in salivary gland tissue from patients with primary Sjögren's syndrome and mouse models.

Results: In the study, 31 human data samples were calculated and finally obtained by the CIBERSORT deconvolution method. The results of immune cell infiltration found that compared with normal human salivary gland tissue, gamma delta T cells were significantly different from naive $CD4^+$ T cells content was significantly increased, while plasma cell content decreased. Principal component analysis (PCA) results suggested differences in immune cell infiltration between pSS patients and normal subjects. At the same time, The ESS model mice was constructed. For the mouse data analysis, we found that the proportion of macrophages increased, while the proportion of $CD4^+$ T cells, B cells and monocytes decreased. After comprehensive analysis, we found that pSS patients and models were smaller the proportion of monocytes in salivary gland tissue of mice decreased, while the proportion of macrophages increased. The infiltration proportions of $CD4^+$ T cells, $CD8^+$ T cells and B cells showed a certain difference.

Conclusions: Through a comprehensive analysis of salivary gland immune infiltration in patients with primary Sjögren's syndrome and model mice, we demonstrate conservation and nonconservation in the mouse and human immune systems at the level of immune cells, helping to explain the primary Regulation of immune mechanisms during the development of Sjögren's syndrome.

Introduction

Primary Sjogren's syndrome (pSS) is a common systemic autoimmune disease. Statistics show that the incidence of women is much higher than that of men, with a ratio of 9–14:1. It is more common in middle-aged and older women[1]. The disease has obvious characteristics, which is manifested as the loss of secretory function caused by the infiltration of lymphocytes in the exocrine glands. Among them, the lacrimal gland and salivary gland dysfunction are the most common [2]. In addition, with the further development of the disease, other organs, such as kidneys, will also suffer different degrees of damage[3]. The pathogenesis of the disease has not yet received a unified opinion, and it may be the immune damage caused by the mutual influence of immune responses, the occurrence and progression of pSS.

Affected by many factors, such as environment, genetics, etc. [4]. The abnormal number of immune cells in the target organs and blood of pSS patients is also an important factor in the occurrence and aggravation of the disease [5] .

Bioinformatics analysis is playing an increasingly important role in biomedical research due to the development of gene chips and next-generation sequencing technologies, and the CIBERSORT algorithm can be used to assess immune cell infiltration in tissues based on gene expression datasets. A large number of studies have used this algorithm to explore the composition of immune cells in disease [6]. RNA-seq enables analysis of the entire transcriptome, thus revealing changes in entire signaling networks and potentially predicting novel genes of significance. RNA-seq has become one of the standard techniques in molecular biology research[7].By using RNA-seq, the sequence of coding RNA or non-coding RNA in cells can be clearly detected, even some small RNAs, and all or part of specific RNAs can also be detected according to the research wishes of scholars. It is worth noting that using this technology allows scholars to obtain gene expression more quickly and conveniently than previous technologies. At present, the extraction of tissue immune environment from transcriptome data has gradually become a research hotspot, but it rarely involves mouse RNA-Seq data. The seq-ImmuCC model is a model developed by scholars in recent years for mouse RNA-Seq data. This model can describe the composition of major immune cells in different tissues or organs from mouse transcriptomic data[8].

Mouse models are invaluable tools for characterizing signaling pathways and biomedical research, and most studies on pSS use mice as model organisms. However, immunological differences between humans and mice are known[9, 10], and translating discoveries in model organisms to human therapy has been challenging[11]. Overall, our study complements research in model organisms to improve translation of laboratory inventions into clinical benefit. In this study, we performed immune infiltration analysis of the pSS human salivary gland CIBERSORT[12]model and the pSS C57BL/6. mouse seq-ImmuCC[8]model, and performed a comprehensive comparison to determine the cellular characteristics of humans and mice, Evolutionary similarities in the human and mouse immune systems are shown. This may provide some insights into the immunomodulatory mechanism of pSS.

Materials And Method

Data Sources

Download the gene expression profile chip dataset of salivary glands of normal human and pSS patients from the gene expression omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/), and obtain the GSE40611 dataset. A total of There were 31 samples, including 16 normal human salivary gland tissues and 15 pSS patients' salivary gland tissues.

Evaluation of immune cell infiltration patterns in salivary gland tissue of patients with pSS

Using perl software, the gene expression data were organized into a gene matrix with the row name of gene name and the column name of sample name. The mRNA expression profile matrix of salivary gland tissue of normal human and pSS patients was corrected by BioManager package and limma package of R language. Deconvolution analysis was performed on the expression matrix of human immune cell subtypes with CIBERSORT, the relative proportions of 22 immune cells in the tissue were calculated, and a P value was obtained for each sample. The samples were screened according to P<0.05, and 16 normal human and Immune cell composition matrix of salivary gland tissue from 15 patients with pSS. Use R language and related packages to draw histograms and immune cell expression heatmaps of each immune cell composition ratio in the two groups of samples, and use the corrplot package in R language to analyze the correlation of immune cells in the salivary glands of pSS patients and draw heatmaps , using the vioplot package in the R language to compare and analyze the proportion of salivary gland immune cells in normal humans and pSS patients, and draw a violin diagram.

Construction of Sjögren's syndrome mouse model

Specific pathogen-free (SPF) 6-week-old female C57BL/6 mice were purchased from VitalLiver Laboratory Animal Technology. All mice were housed at constant temperature and humidity and fed ad libitum. All animal studies were performed in accordance with protocols approved by the Animal Experiment Ethics Review Committee of Anhui University of Traditional Chinese Medicine(Animal Ethics Number: AHUCM-mouse-2021082). Construction of salivary gland protein-induced pSS mouse model[13], the SGs protein emulsion was subcutaneously injected into the dorsal and caudal bases of the mice to establish ESS. At the same time, we regularly detected the salivary flow of the mice at 0, 6, 7, and 8 weeks[14], and combined with the HE staining histopathological score of the mice. To determine the structure of the mouse model[15]. Mice that were unsuccessful in modeling were eliminated, and pSS model mice that exhibited clinical symptoms and significantly decreased salivary flow were screened. Mice were randomly divided into 2 groups: control group (n=3) and ESS group (n=6) for subsequent RNA-seq sequencing.

RNA-seq sequencing

The salivary glands of three control groups and six model groups were selected from the mice for RNA-seq sequencing. The mice in the model group showed significantly reduced salivary secretion and obvious inflammation foci in the pathological section specimens.

RNA from total samples was isolated and purified using TRIzol (Invitrogen, CA, USA) according to the protocol provided by the manufacturer. The quantity and purity of total RNA were then checked with NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). The integrity of RNA was detected by Bioanalyzer 2100 (Agilent, CA, USA) and verified by agarose electrophoresis. Concentration>50ng/μL, RIN value>7.0, OD260/280>1.8, total RNA>1μg to meet downstream experiments. PolyA (polyA)-bearing mRNA was specifically captured by two rounds of purification using oligo(dT) magnetic beads (Dynabeads Oligo (dT), Cat. No. 25-61005, Thermo Fisher, USA). The captured mRNA was fragmented using a Magnesium

Fragmentation Kit (NEBNext® Magnesium RNA Fragmentation Module, Cat. No. E6150S, USA) at high temperature at 94°C for 5-7 minutes. cDNA was synthesized from the fragmented RNA by reverse transcriptase (Invitrogen SuperScript™ II Reverse Transcriptase, Cat. No. 1896649, CA, USA). Then, using E. coli DNA polymerase I (NEB, Cat. No. m0209, USA), and RNase H (NEB, Cat. No. m0297, USA) for double-strand synthesis, these complex duplexes of DNA and RNA were converted into DNA double-strands. dUTP Solution (Thermo Fisher, Cat. No. R0133, CA, USA) was incorporated into the second strand to blunt the ends of the double-stranded DNA. Then, an A base is added to each of its two ends, so that it can be connected with a linker with a T base at the end, and the fragment size is screened and purified by magnetic beads. Digest the second strand with UDG enzyme (NEB, cat. no. m0280, MA, US), and then perform PCR—pre-denaturation at 95°C for 3 minutes, denaturation at 98°C for a total of 8 cycles of 15 seconds each, annealing to 60°C for 15 seconds, Extend at 72°C for 30 seconds, and finally extend at 72°C for 5 minutes to form a library with a fragment size of 300bp±50bp. Finally, we performed paired-end sequencing using an illumina Novaseq™ 6000 (LC Bio Technology CO., Ltd. Hangzhou, China) according to standard procedures in PE150 sequencing mode.

Statistical methods

Statistical analysis R (version 4.0.2) and Bioconductor (https://www.bioconductor.org/) packages were used for statistical analysis. For data sorting, the list deletion method is used for processing, and the entire column is deleted for samples lacking any single value, which is not used for statistical analysis. The corrplot package in R language was used to detect and draw the correlation between different immune cells in the salivary gland tissue of pSS patients; the vioplot package in R language was used to calculate and draw the proportion of different immune cells in the two groups of samples. $P<0.05$ was considered to be statistically significant. We used the newly developed seq-ImmuCC (http://218.4.234.74:3200/immune/) to process the off-camera data according to the methods in the literature[8, 16]. Agreement between relative scores of immune cell types was determined by Pearson's correlation coefficient to measure the degree of linear fit, and root mean square error (RMSE) was used to assess estimation bias. Heatmaps were performed using the 'pheatmap' package of the R software. Statistical analysis was performed using the R package. Results at $P < 0.05$ were considered statistically significant [17].

Results

Analysis of immune cell infiltration in pSS patients and normal subjects

The CIBERSORT deconvolution method is used to calculate and finally obtain 31 credible samples, of which 16 are normal samples and the remaining 15 are pSS samples. The results of immune cell infiltration showed that, as shown in (Figure 1A), compared with the normal group, the T cells CD4 naive, T cells gamma delta, B cells memory and T cells CD4 memory resting in the pSS group were significantly increased, and plasma cells were significantly decreased. (Figure 1B) further shows the infiltration ratio of

22 types of immune cells. (corresponding color modules indicate the type, and size indicates the proportion of the total immune cells).

Human Immune Cell Correlation Analysis

Immune cell correlation analysis showed that the difference between T cells gamma delta and T cells CD4 naive ($r=0.61$, $P<0.05$) as well as between NK cells resting and Mast cells activated ($r= 0.66$, $P<0.05$), there is a significant positive correlation. There was a significant negative correlation between T cells gamma delta and plasma cells ($r=-0.7$, $P<0.05$) (Figure 2A). The violin diagram shows that pSS patients have abundant macrophage infiltration compared with normal people. In addition, compared with normal people, pSS patients have significantly increased content of B cells naive, CD4 memory activated and T cells gamma delta. The content of plasma cells, NK cells resting and monocytes decreased significantly (Figure 2B). Principal component analysis (PCA) based on infiltrating immune cell types revealed discriminative results between pSS and healthy tissue in Figure 2C. The results showed that there were differences in immune cell infiltration between pSS patients and normal subjects (Figure 2C).

Establishment of ESS model

After the C57BL/6 mice in the model group were immunized with allogeneic SG protein, most of the mice developed the clinical symptoms of pSS. At the same time, we could find that the saliva secretion of the mice in the model group decreased significantly from the 6th week after immunization, while the corresponding unimmunized controls did not, and the overall salivary secretion leveled off (Figure 3A). After histological analysis of salivary gland tissue sections of ESS model mice and control mice, we found that 6 weeks after immunization (Figure 3B), the salivary glands of ESS mice suffered more lymphocyte infiltration, and 8 weeks after immunization Afterwards, we found multiple lymphocyte foci and we could intuitively see that the acinus of the mice in the model group was also damaged (Figure 3C), while the above description was not found in the control group. At the same time, we used a scoring system. The infiltration severity was quantified to achieve an intuitive expression effect, and it was found that the histological scores in the pSS model group were significantly higher than those in the control group, and the scores in the model group tended to increase over time (Figure 3D). Combining the above conditions, it can be seen that this model is relatively successful.

Analysis of immune cell infiltration in mice

Based on mouse salivary gland tissue RNA-Seq data, using the seq-ImmuCC model, 10 types of immune cells (B.cells, $CD4^+$ T cells, monocytes, macrophages, neutrophils, $CD8^+$ T cells, NK cells, mast cells, dendritic cells and eosinophils) in salivary gland tissue were analyzed. In all 9 salivary gland tissues, there was abundant macrophage infiltration as shown in (Figure 4A). Cluster analysis showed that the immune cell infiltration of ESS model mice and normal control mice showed obvious inter-group differences (Figure 4B). Immune cell correlation analysis showed that Eosinophils and B.cells ($r=0.95$, $P<0.05$), and Monocytes and B.Cells ($r =0.86$, $P<0.05$) Neutrophils and $CD4^+$ T cells ($r=0.77$, $P<0.05$) Monocytes and Eosinophils ($r=0.69$, $P<0.05$), there was a significant positive correlation. While

Macrophages and B.Cells (r=−0.78, P<0.05), Macrophages and eEosinophils (r=−0.6, P<0.05) , Monocytes and Macrophages (r=−0.89, P<0.05) had a significant negative correlation (Figure 4C). The violin diagram shows that compared with the control mice, in the salivary glands of the ESS model mice, the proportions of macrophages and $CD8^+$ T cells were significantly increased, while the proportions of $CD4^+$ T cells, Monocytes, and B.cells were significantly decreased (Figure 4D).

Comprehensive analysis of immune cell infiltration in pSS patients and ESS model mice

Through the analysis of the immune infiltration of pSS patients and ESS model mice, we found that ESS model mice have certain similarities and differences in the composition of human immune cells. For human data, we found that T cells gamma delta, T cells CD4 naive, B cells naive, M1 Macrophages and activated memory $CD4^+$ T cells were significantly increased, while plasma cells, NK cells Resting, the content of Monocytes decreased significantly (Figure 2B). For the data analysis of the model mice, we found that the number of Macrophages increased significantly, and the proportion of $CD8^+$ T cells also increased, while the proportion of $CD4^+$ T cells, B.cells and Monocytes decreased (Figure 4D). Combining the two results, the proportion of Monocytes (p=0.001) in pSS patients and in the salivary gland tissue of model mice (p=0.0489) was reduced. Macrophages (p=0.0476) were significantly increased in model mice, while M1 macrophages (p=0.028) were significantly increased in pSS patients, while M0 macrophages (p=0.173) and M2 macrophages (p= 0.207) the increase is not obvious. The proportion of $CD8^+$ T cells (p=0.0238) only increased in model mice. The contents of $CD4^+$ T cells (p=0.0262) and B.cells (p=0.0431) in the tissue of the model mice were decreased to varying degrees, but they showed an upward trend in pSS patients.

Discussion

pSS is an autoimmune disease common in middle-aged and elderly women that targets exocrine glands such as salivary glands and may involve multiple organs and systems. At present, many studies have shown that the proportion of immune cells in patients with pSS is different from that in normal people, which is also an important factor in the occurrence and aggravation of the disease. High-throughput analytical techniques such as transcriptomics and expression microarray analysis allow us to gain insight into changes in immune cell composition, and a unique feature of this study is the correlation of human gene expression data with those of Sjögren's syndrome-susceptible C57BL/6 Comprehensive analysis of the data. Allows us to characterize the cells in human and mouse models and may provide some insight into the mechanisms of pSS immune regulation.

In this study, in the human research, we downloaded the gene expression profile chip dataset of salivary glands of normal human and pSS patients from GEO, namely the GSE40611 dataset, used R package to rectify the data, and used CIBERSORT to analyze the data. Expression matrices of human immune cell subtypes were deconvoluted and finally plotted using R software. In mouse studies, we first constructed an ESS mouse model using homologous mouse salivary proteins that highly recapitulates the key features of human pSS, and identified changes in saliva volume and lymphocyte infiltration in salivary

gland tissue. Determine the success of the model. Mouse salivary glands were collected and preserved during the process. After that, we used the collected mouse salivary gland tissue as a sample, sequenced the tissue by RNA-Seq technology, and the related sequencing results were processed by seq-ImmuCC, and R software was used to generate the required related charts to conduct a comprehensive analysis of the above data, Summarizing the cellular characteristics between the two has expanded a new horizon for the pSS-related regulatory mechanism.

In this study, through the analysis of immune infiltration, we found that mouse and human immune cells have certain common features. For human data, we found that T cells gamma delta and T cells CD4 naive was positively correlated with plasma cells, and we could find naive B cells naive, CD4 memory activated, T cells gamma delta was significantly increased. And Plasma cells, NK cells resting, Monocytes, the content decreased significantly. Furthermore, PCA results suggest that immune cell infiltration can be used to differentiate pSS patients from normal individuals. For mouse data analysis, we found that Macrophages were negatively correlated with B.Cells, Eosinophils, Monocytes, and the number of macrophages increased. The trend of $CD4^+$ T cells, Monocytes, and B.cells decreased significantly.

B lymphocytes are generated by differentiation of hematopoietic stem cells, mature in the bone marrow, and finally activate in secondary lymphoid organs. It has been suggested that the increased frequency of autoreactive antibody expression by mature naive B cells and impaired peripheral B cell tolerance in patients with pSS may be involved in the pathogenesis of pSS [18]. B10 cells are a subset of B cells that can produce IL-10, which play an important role in maintaining immune stability mainly through the negative immune regulation function of IL-10. In the process of inflammatory response, B10 cells affect the occurrence and development of pSS through multiple factors, such as inducing T cell anergy or Treg expansion during antigen presentation, or directly inhibiting Th1 and Th17 through IL-10 to alleviate the disease[19]. Some studies have also found that B10 plays a key role in regulating Tfh cell responses. Adoptive transfer of B10 cells can effectively inhibit Tfh cell responses and attenuate the histopathological score of ESS mice [20]. In sexually transmitted diseases, the frequency of cells is also significantly reduced, the inhibitory cytokines secreted by them are also reduced to a certain extent, and the number of B10 cells is negatively correlated with the progression of the disease[21]. This is consistent with our finding that the number of naive B cells increased while the number of B cells decreased.

Macrophages are innate immune cells and play a myriad of important roles, such as host defense, tissue homeostasis, and regulation of inflammatory responses[22, 23]. Macrophages are innate immune cells and play a myriad of important roles, such as host defense, tissue homeostasis, and regulation of inflammatory responses[24]. And macrophages are an important part of B cells. Many studies have shown that macrophages play an important role in the pathological damage of salivary and lacrimal glands. Macrophages in the salivary glands are obviously pathogenic and can secrete related cytokines, and patients with significant macrophage infiltration in the salivary glands have a higher degree of salivary gland swelling[25]. This is consistent with the increased numbers of macrophages in our results.

$CD8^+$ T lymphocytes are a complex group of lymphocytes with different phenotypes. Studies have shown that activated $CD8^+$ T lymphocytes aggregate around apoptotic acinar epithelial cells, and $CD8^+$ T cells infiltrating glands show cytotoxicity. Not affected by $CD4^+$ T cells[26]. it is worth noting that as the disease progresses, the proportion of $CD8^+$ T cells in pSS patients is significantly increased compared with normal people[27], which is consistent with our results of. Memory $CD4^+$ T cells are highly associated with autoimmune diseases due to their long-lived properties, efficient responses to antigens, and the potential to mediate recurrent autoimmune responses[28]. However, many key questions about the potential contribution of memory $CD4^+$ T cells to autoimmune disease remain unanswered. Some studies have shown that the relative proportion of memory $CD4^+$ T in patients with pSS compared with normal controls is increased[29]; this is consistent with our results of human immune infiltration, but the specificity of memory T cells and pSS is different. The interrelationships are still not fully understood. In addition, some scholars have found that the reduction of lymphocytes in pSS patients is related to the premature senescence of naive $CD4^+$ T cells in the body, which also explains the reduction of $CD4^+$ T lymphocytes in the mouse data [30]. Determining the immunobiological roles of $CD4^+$ T and $CD8^+$ T cells in pSS is critical for the development of targeted therapies for pSS driven by $CD4^+$ or $CD8^+$ T cells. Gamma delta T cells (T cells gamma delta) are γ/δ^+ T cells, accounting for about 2–5% of peripheral blood T lymphocytes, mainly distributed in mucosa-associated lymphoid tissues, and their immune functions are between innate immunity and adaptive immunity. Between immunity, plays a role in tissue homeostasis and infection monitoring [31]. It has been reported that the proportion of activated cells in γ/δ^+ T cell subsets in peripheral blood of pSS patients is significantly higher than that of controls, and the frequency of activated cells is related to the disease course of pSS patients [32], which is consistent with our results. Consistently, this suggests that this T cell subset may play a role in the pathological immune response encountered in pSS.

On the other hand, monocytes have been studied relatively less than lymphocytes, but they are known to be involved in the pathogenesis of pSS. Indeed, expanding data suggest that infiltrating monocytes and macrophages are phenotypically altered in autoimmune disease and have been implicated in both mice and humans[33, 34]. In humans, monocytes are divided into three subtypes, "classical" monocytes, "intermediate" monocytes, and "pro-inflammatory", based on the relative surface expression of the lipopolysaccharide (LPS) coreceptor CD14 and the FCγIII receptor CD16 monocytes. Several reports suggest that the primary function of most circulating monocytes is the phagocytosis of cellular debris, pathogens, and other external factors[35, 36], in a manner that does not involve the release of inflammatory mediators; however, monocytes in patients with pSS have been observed Cells are inefficient in the clearance of apoptotic cells, which is also associated with the production of pro-inflammatory cytokines such as IL-6, TNF-α, IL-10, transforming growth factor (TGF)-β and interferon (IFN)- α[37–39].

However, the limitation of this study is that the seq-ImmuCC model only covers 10 cell types. For example, gamma delta T cells, which play an important role in the immune system, are not included, and further improvement is needed in the future. In addition, the human part is based on bioinformatic immune

infiltration analysis of transcriptomic signatures from public datasets, which may not correspond to the actual situation, and further studies are needed to confirm the findings observed in human biopsies.

Conclusions

In this study, we performed immune infiltration analysis in pSS humans and model mice, and predicted abnormal disease-specific immune infiltration patterns to reveal the underlying immune pathogenesis of pSS. We found that monocyte levels were reduced in both pSS patients and model mice. While further experiments are needed to demonstrate that these cells perform tissue damage in pSS, our results suggest that this may be the case, providing clues for disease-modifying immunotherapies by targeting specific immune cells.

Declarations

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Author contributions

Xingxing Huo conceived and designed the experiments, performed the experiments, approved the final version, analyzed the data. Yajun Qi and Hongxiao Gong contributed to the drafting of the submitted article and the accuracy of the data analysis. Xiaoting Qiu and Hongxiao Gong contributed to the acquisition of reagents, materials and analysis tools. Yajun Qi and Hongxiao Gong contributed to the analysis and interpretation of the data. Xingxing Huo, and Ling Zhu contributed to perform the validation experiment and revise the manuscript.

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Data availbility statement

The datasets generated and analysed during the current study are available in the NCBI repository(https://dataview.ncbi.nlm.nih.gov/object/PRJNA839202?reviewer=kn6p7afmgfcp69v6f0spijtalb).

Ethics approval and consent to participate

All animal studies were performed in accordance with ARRIVE guidelines and other relevant guidelines/regulations. Mouse normal salivary gland tissue was obtained from 6-week-old female C57BL/6 mice. A salivary gland protein induction model was constructed using female mice. The Animal

Experiment Ethics Review Committee of Anhui University of Traditional Chinese Medicine approved all animal experiments in this study (AHUCM-mouse-2021082).

Consent for publication

Not applicable.

Conflict of Interests

The authors declare that they have no conflict of interest.

Animal Experimental Research Statement

The authors confirm the study is reported in accordance with ARRIVE guidelines.

References

1. Bjrk A, Mofors J, Wahren-Herlenius M: **Environmental factors in the pathogenesis of primary Sjgren's syndrome**. *Journal of Internal Medicine* 2020, **287**(5):475-492.
2. Bjordal O, Norheim KB, Rodahl E, Jonsson R, Omdal R: **Primary Sjogren's syndrome and the eye**. *Survey Of Ophthalmology* 2020, **65**(2):119-132.
3. Lefaucheur J-P, Sene D, Oaklander AL: **Primary Sjogren's Syndrome**. *New England Journal Of Medicine* 2018, **379**(1):96-96.
4. Bombardieri M, Argyropoulou OD, Ferro F, Coleby R, Baldini C: **One year in review 2020: pathogenesis of primary Sjgren's syndrome**. *Clinical and Experimental Rheumatology* 2020, **38 Suppl 126**(4):3-9.
5. Torgashina AV, Vasilyev VI: **The efficacy of rituximab in the therapy of neuromyelitis optica in a patient with Sjogren's syndrome: Case-report and literature review**. *Terapevticheskii Arkhiv* 2018, **90**(5):76-80.
6. Zhao Y, Xia Z, Lin T, Yin Y: **Significance of hub genes and immune cell infiltration identified by bioinformatics analysis in pelvic organ prolapse**. *PeerJ* 2020, **8**(5):e9773.
7. Alda-Catalinas C, Eckersley-Maslin MA, Reik W: **Pooled CRISPR-activation screening coupled with single-cell RNA-seq in mouse embryonic stem cells**. *STAR Protocols* 2021, **2**(2):100426.
8. Chen Z, Quan L, Huang A, Zhao Q, Yao Y, Yuan X, Qin S, Shang J, Yinyin B, Qin XF: **seq-ImmuCC: Cell-Centric View of tissue transcriptome Measuring Cellular Compositions of Immune Microenvironment From Mouse RNA-seq data**. *Frontiers in Immunology* 2018, **9**:1286.
9. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L *et al*: **Genomic responses in mouse models poorly mimic human inflammatory diseases**. *Proc Natl Acad Sci U S A* 2013, **110**(9):3507-3512.
10. Shay T, Jojic V, Zuk O, Rothamel K, Puyraimond-Zemmour D, Feng T, Wakamatsu E, Benoist C, Koller D, Regev A *et al*: **Conservation and divergence in the transcriptional programs of the human and mouse immune systems**. *Proc Natl Acad Sci U S A* 2013, **110**(8):2946-2951.

11. J R: **Animal models: Not close enough**. *Nature* 2012, **484**:S 9.
12. Shan D, Guo X, Yang G, He Z, Li G: **Integrated Transcriptional Profiling Analysis and Immune-Related Risk Model Construction for Intracranial Aneurysm Rupture**. *Frontiers in neuroscience* 2021, **15**:613329.
13. Gao Y, Chen Y, Zhang Z, Yu X, Zheng J: **Recent Advances in Mouse Models of Sjgren's Syndrome**. *Frontiers in Immunology* 2020, **11**.
14. Lau, Chak, Sing, Ko, King-Hung, Lin, Xiang, Cao, Xuetao, Jiao: **Th17 cells play a critical role in the development of experimental Sjogren's syndrome**. *Annals of the Rheumatic Diseases: A Journal of Clinical Rheumatology and Connective Tissue Research* 2015.
15. Chisholm DM, Waterhouse JP, Mason DK: **Lymphocytic sialadenitis in the major and minor glands: a correlation in postmortem subjects**. *Journal of Clinical Pathology* 1970, **23**(8):690-694.
16. Wu, Aiping, Qin, Xiao-Feng F, Chen, Ziyi, Huang, Anfei: **Inference of immune cell composition on the expression profiles of mouse tissue**. In*: 2017*.
17. Wu D, Zhou P, Cao F, Lin Z, Zhou X: **Expression Profiling and Cell Type Classification Analysis in Periodontitis Reveal Dysregulation of Multiple lncRNAs in Plasma Cells**. *Frontiers in Genetics* 2020, **11**:382.
18. Glauzy S, Sng J, Bannock JM, Gottenberg JE, Korganow AS, Cacoub P, Saadoun D, Meffre E: **Defective Early B Cell Tolerance Checkpoints in Sjogren's Syndrome Patients**. *Arthritis Rheumatol* 2017, **69**(11):2203-2208.
19. Bird AK, Meednu N, Anolik JH: **New insights into B cell biology in systemic lupus erythematosus and Sjögren's syndrome**. *Current Opinion in Rheumatology* 2015, **27**(5).
20. Lin X, Wang X, Xiao F, Ma K, Liu L, Wang X, Xu D, Wang F, Shi X, Liu D: **IL-10-producing regulatory B cells restrain the T follicular helper cell response in primary Sjgren's syndrome**. *Cell Mol Immunol* 2019.
21. Ben Nasr M, Usuelli V, Seelam AJ, D'Addio F, Abdi R, Markmann JF, Fiorina P: **Regulatory B Cells in Autoimmune Diabetes**. *Journal of immunology (Baltimore, Md : 1950)* 2021, **206**(6):1117-1125.
22. Watanabe S, Alexander M, Misharin AV, Budinger GRS: **The role of macrophages in the resolution of inflammation**. *J Clin Invest* 2019, **129**(7):2619-2628.
23. Wynn TA, Chawla A, Pollard JW: **Macrophage biology in development, homeostasis and disease**. *Nature* 2013, **496**(7446):445-455.
24. Pan Y, Yu Y, Wang X, Zhang T: **Tumor-Associated Macrophages in Tumor Immunity**. *Front Immunol* 2020, **11**:583084.
25. Manoussakis MN, Boiu S, Korkolopoulou P, Kapsogeorgou EK, Kavantzas N, Ziakas P, Patsouris E, Moutsopoulos HM: **Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjogren's syndrome: correlation with certain features of immune hyperactivity and factors associated with high risk of lymphoma development**. *Arthritis Rheum* 2007, **56**(12):3977-3988.

26. Wang, Xiaofang, Barr, Jennifer, Y., Lieberman, Scott, M., Meyerholz, David: **CD8 T cells contribute to lacrimal gland pathology in the nonobese diabetic mouse model of Sjogren syndrome**. *Immunology and Cell Biology* 2017, **95**(8):684-694.
27. Zhou H, Yang J, Tian J, Wang S: **CD8+ T Lymphocytes: Crucial Players in Sjgren's Syndrome**. *Frontiers in Immunology* 2021, **11**.
28. Raphael I, Joern RR, Forsthuber TG: **Memory CD4(+) T Cells in Immunity and Autoimmune Diseases**. *Cells* 2020, **9**(3):23.
29. Joachims ML, Leehan KM, Dozmorov MG, Georgescu C, Pan ZJ, Lawrence C, Marlin MC, Macwana S, Rasmussen A, Radfar L *et al*: **Sjogren's Syndrome Minor Salivary Gland CD4(+)Memory T Cells Associate with Glandular Disease Features and Have a Germinal Center T Follicular Helper Transcriptional Profile**. *J Clin Med* 2020, **9**(7):17.
30. Fessler J, Fasching P, Raicht A, Hammerl S, Weber J, Lackner A, Hermann J, Dejaco C, Graninger WB, Schwinger W *et al*: **Lymphopenia in primary Sjogren's syndrome is associated with premature aging of naive CD4+T cells**. *Rheumatology* 2021, **60**(2):588-597.
31. Ribot JC, Lopes N, Silva-Santos B: **gamma delta T cells in tissue physiology and surveillance**. *Nat Rev Immunol* 2021, **21**(4):221-232.
32. Ichikawa Y, Shimizu H, Yoshida M, Takaya M, Arimori S: **T cells bearing gamma/delta T cell receptor and their expression of activation antigen in peripheral blood from patients with Sjgren's syndrome**. *Clinical & Experimental Rheumatology* 1991, **9**(6):603-609.
33. Barrera Garcia A, Gomez-Puerta JA, Arias LF, Burbano C, Restrepo M, Vanegas AL, Munoz CH, Rojas M, Gonzalez LA, Vasquez G: **Infiltrating CD16(+) Are Associated with a Reduction in Peripheral CD14(+)CD16(++) Monocytes and Severe Forms of Lupus Nephritis**. *Autoimmune Dis* 2016, **2016**:9324315.
34. Mikolajczyk TP, Osmenda G, Batko B, Wilk G, Krezelok M, Skiba D, Sliwa T, Pryjma JR, Guzik TJ: **Heterogeneity of peripheral blood monocytes, endothelial dysfunction and subclinical atherosclerosis in patients with systemic lupus erythematosus**. *Lupus* 2016, **25**(1):18-27.
35. Mukherjee R, Kanti Barman P, Kumar Thatoi P, Tripathy R, Kumar Das B, Ravindran B: **Non-Classical monocytes display inflammatory features: Validation in Sepsis and Systemic Lupus Erythematous**. *Sci Rep* 2015, **5**:13886.
36. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ *et al*: **Nomenclature of monocytes and dendritic cells in blood**. *Blood* 2010, **116**(16):e74-80.
37. Byrne JC, Ni Gabhann J, Lazzari E, Mahony R, Smith S, Stacey K, Wynne C, Jefferies CA: **Genetics of SLE: functional relevance for monocytes/macrophages in disease**. *Clin Dev Immunol* 2012, **2012**:582352.
38. Li Y, Lee PY, Reeves WH: **Monocyte and macrophage abnormalities in systemic lupus erythematosus**. *Arch Immunol Ther Exp (Warsz)* 2010, **58**(5):355-364.

39. Sule S, Rosen A, Petri M, Akhter E, Andrade F: **Abnormal production of pro- and anti-inflammatory cytokines by lupus monocytes in response to apoptotic cells**. *PLoS One* 2011, **6**(3):e17495.

Figures

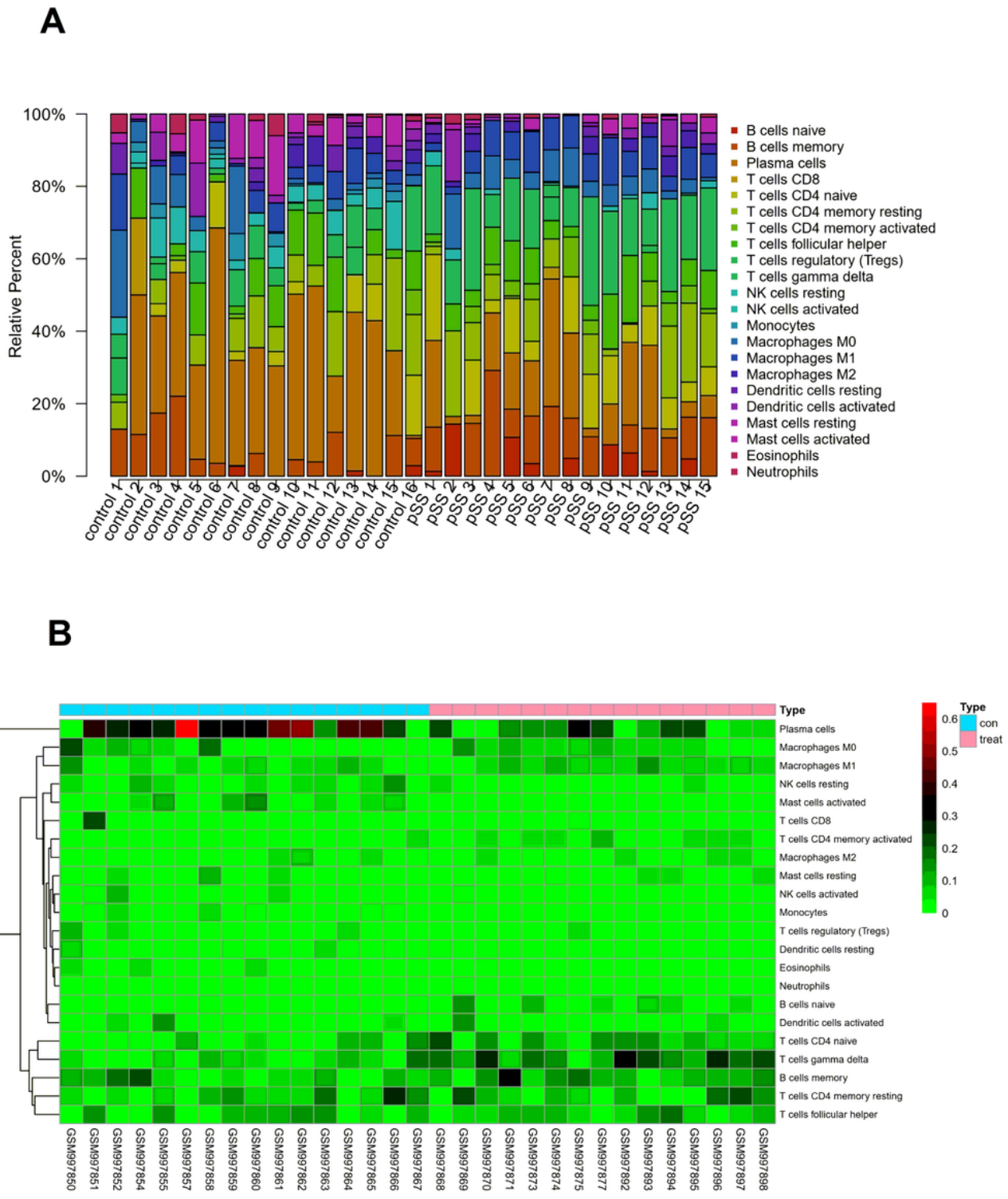

Figure 1

Results of salivary gland immune cell infiltration in pSS patients and normal individuals. (A) Histograms of the proportions of 22 immune cells in the salivary glands of pSS patients and normal individuals. (B) Heat map of the proportions of 22 immune cells in the salivary glands of pSS patients and normal individuals.

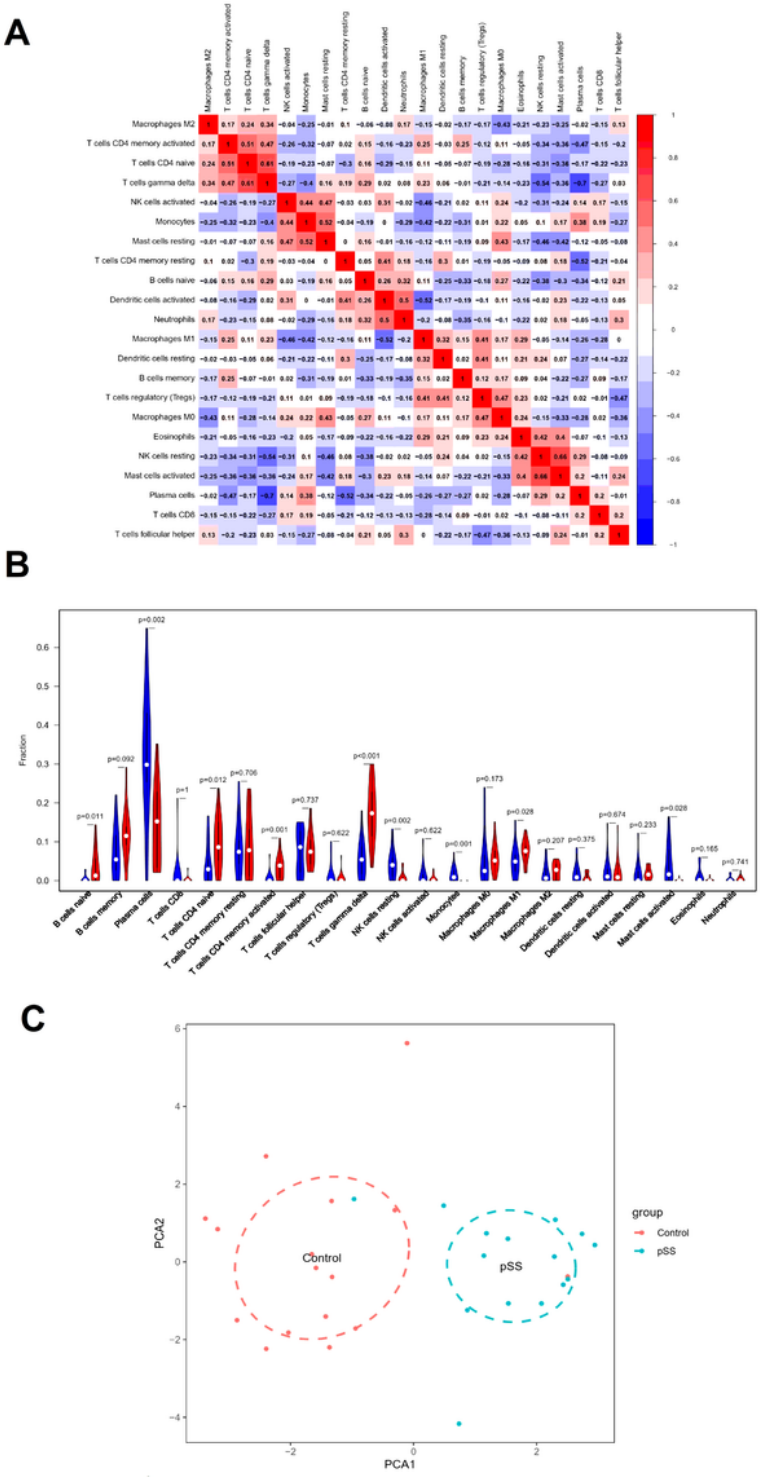

Figure 2

Correlation of salivary gland immune cells between pSS patients and normal people. (A) Correlation between immune cell subsets. There was a significant negative correlation between T cells gamma delta and Plasma cells. (B) Comparison of 22 immune cell subsets in the salivary glands of pSS patients and normal humans. The proportion of T cells gamma delta and M1 Macrophage infiltration was higher in pSS patients. (C) Principal component analysis plot of salivary gland immune cell infiltration in pSS patients and normal individuals. Note: blue is normal group; red is pSS group

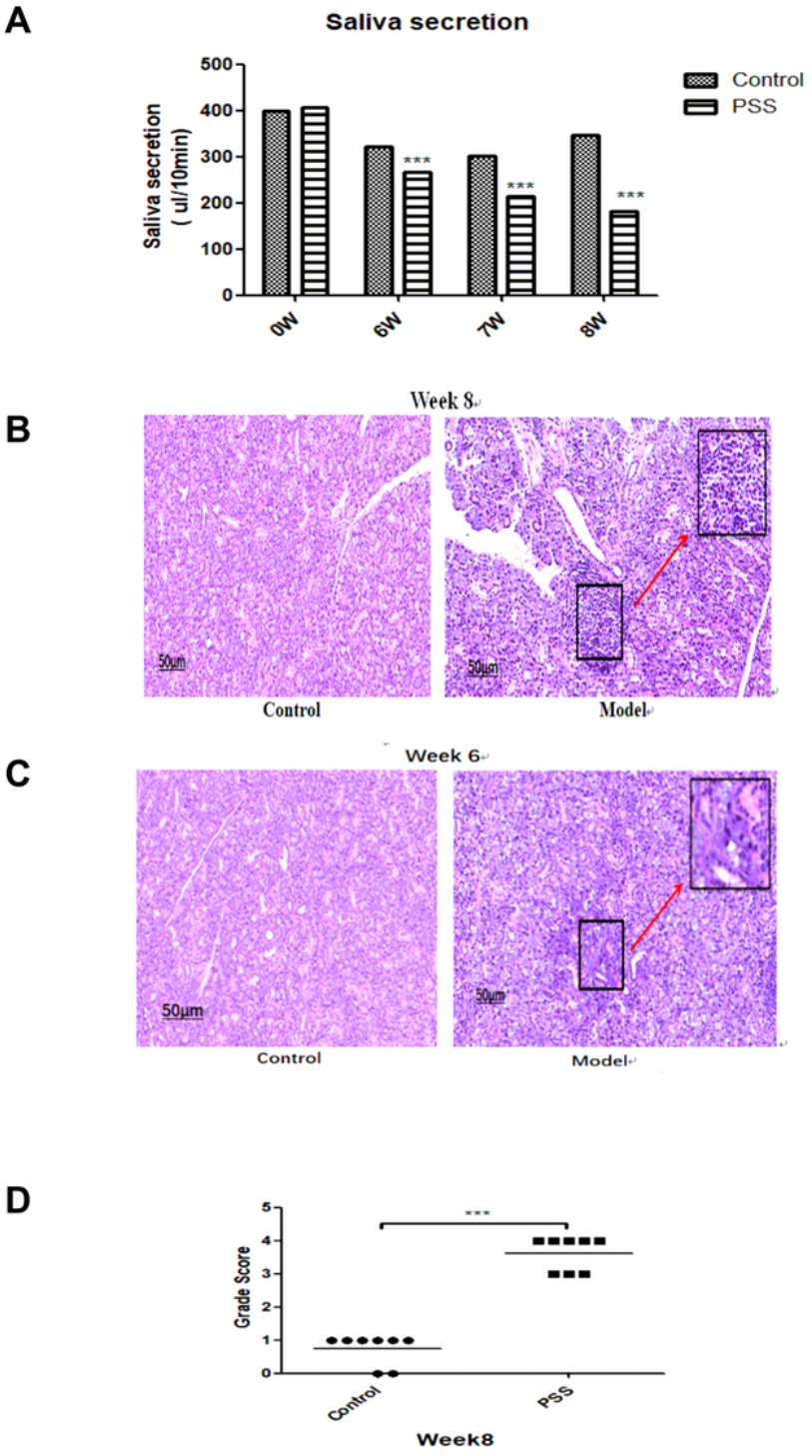

Figure 3

Mouse modeling results. (A) The saliva secretion of ESS model mice was significantly lower than that of the normal group. Measured at weeks 6, 7 and 8. (χ±s, n=8), *p<0.05 vs control. (B) Histological observation of salivary gland injury at week 6 in pSS mice and controls. (C) Histological observation of salivary gland damage in pSS mice and control group at week 8. (D) Histological scoring of salivary gland lesions in pSS mice and controls (χ±s, n = 8), *p < 0.05 versus controls.

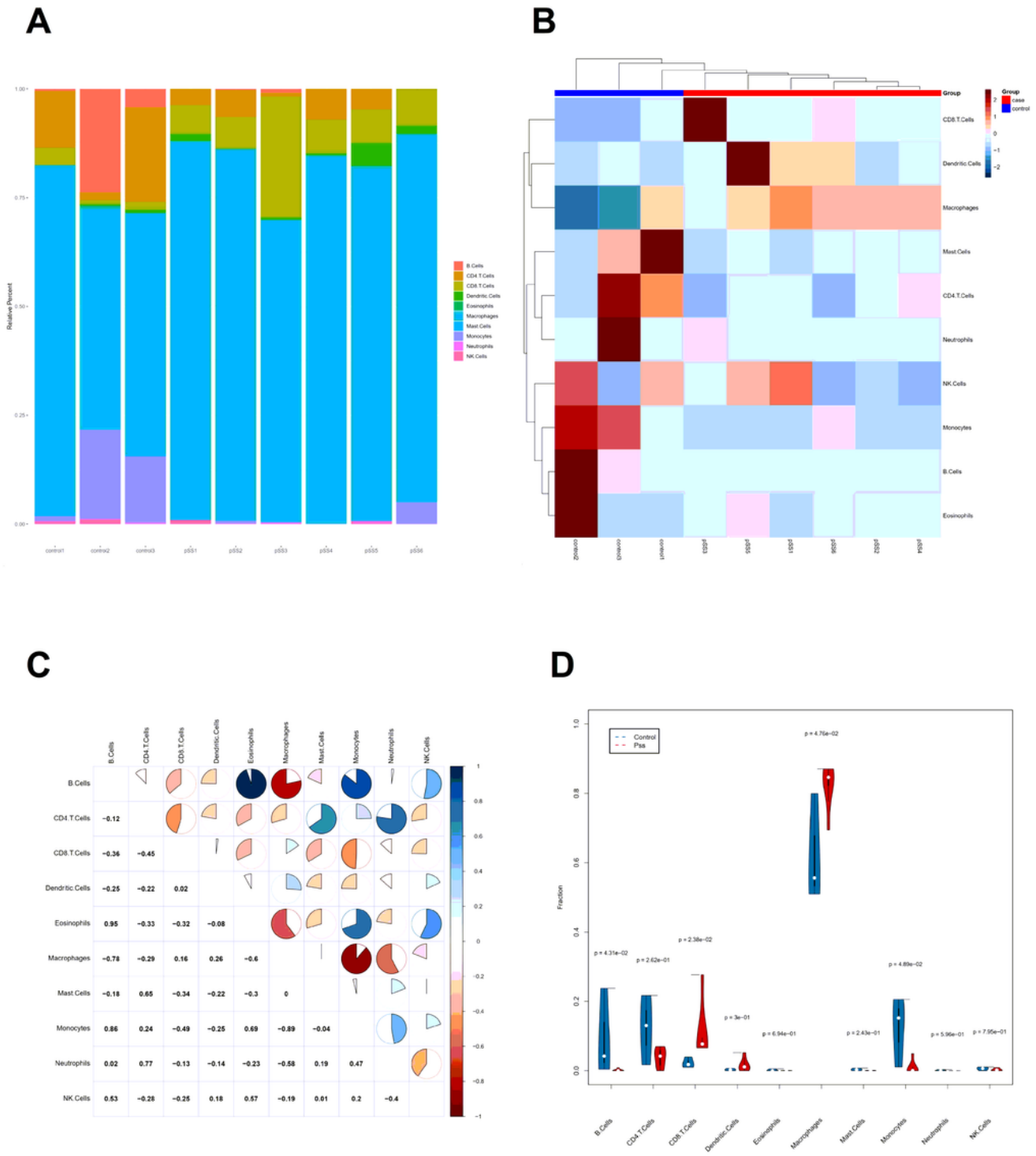

Figure 4

Results of infiltration of salivary gland immune cells in ESS mice. (A) The composition of 10 immune cells in salivary gland tissue based on ImmuCC assay (B) The clustering heat map of salivary gland immune cells in ESS model mice and control mice. (C) Correlations between immune cell subsets. There was a negative correlation between Monocytes and Macrophages. (D) Comparison of infiltrating immune cell subsets in salivary gland tissue of ESS model mice and control mice. The proportion of Macrophage infiltration was higher in ESS model mice. Macrophages are increased in salivary gland tissue of ESS lesions. Note: blue is normal group; red is ESS group.