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Bioinformatic analysis and experimental verification reveal expansion of monocyte subsets with an interferon signature in systemic lupus erythematosus patients

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Abstract

Background Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterized by chronic inflammation and multi-organ damage. A central factor in SLE pathogenesis is the excessive production of type I interferon (IFN-I), which drives immune dysregulation. Monocytes, key components of the immune system, significantly contribute to IFN-I production. However, their specific roles in SLE remain incompletely understood.

Methods This study utilized bioinformatics and statistical analyses, including robust rank aggregation (RRA), DESeq2, and limma, to analyze transcriptome data from peripheral blood mononuclear cells (PBMCs) and monocytes of SLE patients and healthy controls. Single-cell RNA sequencing (scRNA-seq) data were processed using the Seurat R package to identify and characterize monocyte subsets with a strong IFN-driven gene signature. Flow cytometry was employed to validate key findings, using markers such as CD14, SIGLEC1, and IRF7 to confirm monocyte subset composition.

Results Our research has found that monocytes in SLE undergo IFN-driven transcriptional reprogramming, with the upregulation of key interferon signature genes (ISGs), forming the SLE-Related Monocyte Signature (SLERRAsignature). Moreover, the composition of mononuclear phagocyte subsets in SLE patients changes, with an increase trend in the proportion of the CD14Mono8 subset in the flare group. The differentially expressed genes (DEGs) in 13 mononuclear phagocyte subsets of SLE are mainly ISGs, and the expression of ISGs is higher in severe patients. We identified SIGLEC1^{+}IRF7^{+} monocytes among these subsets and for the first time discovered this group of cells in the peripheral blood of healthy individuals. In SLE, the enrichment score of the gene set representing SIGLEC1^{+}IRF7^{+} monocytes is positively correlated with the severity of SLE. Finally, flow cytometry confirmed that the frequency of CD14^{+}SIGLEC1^{+}IRF7^{+} monocytes in PBMCs was higher in SLE compared with healthy controls.

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Conclusions Our study found that the expansion of IFN-I-producing monocyte subsets, particularly the $CD14^{+}SIGLEC1^{+}IRF7^{+}$ subset, plays a crucial role in SLE pathogenesis. This subset may serve as a potential biomarker and therapeutic target for managing SLE.

Keywords Systemic lupus erythematosus, Single-cell RNA sequencing, Type I interferon, Interferon-stimulated genes, $CD14^{+}SIGLEC1^{+}IRF7^{+}$ monocytes

Introduction

SLE is a multifaceted autoimmune disorder that presents significant challenges in diagnosis, therapy, and understanding of its pathophysiology. Characterized by chronic inflammation and widespread tissue damage, SLE can affect virtually every organ system, leading to a broad spectrum of clinical manifestations [1–3]. These range from relatively mild symptoms, such as fatigue and joint pain, to severe, life-threatening conditions including lupus nephritis (LN), central nervous system and cardiovascular complications. The heterogeneity of the disease, both in clinical presentation and underlying immune dysregulation, complicates management and necessitates a deeper understanding of the molecular mechanisms driving the disease [4–6].

Among the various cytokines, IFN-I is particularly central to the pathogenesis of SLE. IFN-I, which includes a family of cytokines such as IFN-α and IFN-β, has robust antiviral properties and shapes the immune response. In SLE, the excessive production of IFN-I results in the aberrant activation of monocytes and other immune cells, exacerbating the chronic inflammation and autoimmunity [7–9]. Previous research has found that monocytes, influenced by IFN-I, experience transcriptional reprogramming that enhances their capacity to generate inflammatory cytokines, present antigens, and engage with other immune cells, thereby amplifying the autoimmune response [10]. In addition, activated monocytes can also produce IFN-I on their own, creating an adverse positive feedback pathway that exacerbates the severity of SLE [8, 11]. Therefore, this IFN-I-related immune activation is closely associated with clinical manifestations in SLE patients, especially in PBMCs. A large proportion of SLE patients exhibit upregulated ISGs in their PBMCs [12, 13]. The upregulated ISGs within PBMCs are referred to as "IFN signature" and have played a significant role in the diagnosis and assessment of SLE [12, 13]. This signature serves as both an indicator of disease activity and an active participant in the disease's process [14]. Several studies of ISGs have reported their involvement in SLE, such as NLRP3 [15–17] and GBPs [18, 19].

Despite the well-documented role of IFN-I in SLE, the specific contributions of different monocyte subsets to disease progression remain poorly understood. Monocytes are not a homogeneous population; they consist of various subsets with distinct functions and phenotypes. These include classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+}CD16^{++}$) monocytes [14], each of which may play different roles in health and disease [14, 20]. Recent advances in scRNA-seq have revealed even greater heterogeneity within these subsets, uncovering previously unrecognized populations that may contribute to the pathogenesis of autoimmune diseases like SLE [13, 21].

The pathogenesis of SLE is complex, and scRNA-seq provides a new way to study it. Wang et al. showed that the number of peripheral blood monocytes in SLE patients increased and the expression of IRF1 was high, which promoted the secretion of a variety of pro-inflammatory factors and broke the immune balance [22]. Sarin et al. found that vaccine-induced monocyte population levels were reduced after vaccination in SLE patients, affecting innate immunity [23]. Honado ost et al. compared SLE and type 1 diabetes and found that C-monocytes were enriched in both, but there were also differences [24]. In summary, monocytes are of great significance in the pathogenesis of SLE and are closely related to immune disorders and disease progression. It provides key clues for revealing the pathogenesis of SLE and developing new therapies.

This work seeks to fill the information gap about the unique functions of monocyte subsets in SLE by utilizing advanced scRNA-seq and bulk RNA-seq methods. These methodologies provide a high-resolution analysis of the transcriptome landscape of monocytes in SLE, elucidating the molecular pathways and gene expression levels that define various subsets. We aim to elucidate the function of monocyte subsets by exhibiting a robust IFN-driven gene signature in the pathogenesis of SLE and investigate their potential as biomarkers and therapeutic targets.

Materials and methods

Integrated analysis of multiple transcriptome data

There are more than a dozen transcriptome expression profiles of peripheral blood from SLE patients. In order to effectively integrate and analyze these data sets and find DEGs in the transcriptome of peripheral blood from SLE patients, RRA method was used to further integrate the DEGs derived from each expression profile data [25]. To find the highly expressed genes in SLE patients by RRA method, we first sorted the DEGs obtained from each expression profile data by Log2 fold change (Log2FC) from large to small, and then used the

RRA algorithm package to re-calculate the differential expression degree of each gene after ranking by differential expression degree. Because RRA integrates multiple transcriptome data based on the rank of the degree of differential expression, the results of integration analysis cannot be affected by the specific value of gene expression, so it can effectively integrate transcriptome data using different detection techniques. (The data integration process throughout the study can be seen in Supplementary Fig. 1.)

Table 1 The transcriptome data of SLE and healthy control monocytes

Source	samples	Analytical technique	clinical characteristics
GSE131525	3 SLE patients and 3 healthy controls	RNA-seq	No special explanation
GSE120442	5 SLE patients and 5 healthy controls	RNA-seq	No special explanation
GSE55447	42 SLE patients and 10 healthy controls	Microarray	Peripheral blood was collected from 21 African-American (AA) and 21 European-American (EA) SLE patients, 5 AA controls, and 5 EA controls.
GSE53419	9 SLE patients and 8 healthy controls	RNA-seq	No special explanation
GSE50395	6 SLE patients and 3 healthy controls	Microarray	3 SLE patients with antiphospholipid syndrome
GSE46907	5 SLE patients and 5 healthy controls	Microarray	The sample consisted of 5 healthy controls and 5 children with SLE
GSE38351	14 SLE patients and 12 healthy controls	Microarray	No special explanation
GSE37356	20 SLE patients and 14 healthy controls	Microarray	Subjects also underwent laboratory and imaging studies of the coronary arteries, carotid arteries, and aorta to evaluate for subclinical atherosclerosis.
E-MTAB-2713	58 SLE patients and 68 healthy controls	Microarray	No special explanation
E-MTAB-145	11 SLE patients and 23 healthy controls	Microarray	No special explanation

Analysis of transcriptome data

We utilized several publicly accessible datasets for our analysis, which were downloaded from the Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/), 10x Genomics Database (www.10xgenomics.com/) and ArrayExpress Database (https://www.ebi.ac.uk/biostudies/arrayexpress). The monocyte transcriptome data from SLE patients and healthy controls (Table 1) were employed for RRA analysis of crucial genes that are highly expressed in SLE. The scRNA-seq data from healthy controls and SLE patients with different clinical types (Supplementary Table 1) were employed to analyze the composition changes of mononuclear phagocytes in the peripheral blood of SLE patients. The scRNA-seq data from autoimmune diseases, cancers, and healthy controls (Supplementary Table 2) were employed to identify and analyze the genetic signatures of mononuclear phagocyte subsets. The transcriptome data of whole blood cells or PBMCs from SLE patients and healthy controls included 14 datasets (Supplementary Table 3) to analyze transcriptome expression differences.

scRNA-seq analysis and cell type identification

The scRNA-seq data were processed using the Seurat R package (version 3.0) [26], which is currently a mainstream and widely used scRNA-seq analysis tool. Quality control was mainly based on the number of feature genes in each cell and the proportion of mitochondrial genes. To reveal the changes of mononuclear phagocyte subsets in patients with SLE by analyzing scRNA-seq of peripheral blood, the number of feature genes measured in each cell must exceed 1000, the proportion of mitochondrial genes must be less than 10%, and mononuclear phagocytes of each sample must exceed 500. To obtain high-quality scRNA-seq to unveil the precise features of the subsets of peripheral blood mononuclear phagocytes, the number of feature genes measured in each cell must exceed 1500, and the proportion of mitochondrial genes must be less than 15%. We used the SCTransform function in Seurat to integrate scRNA-seq data from different samples. This method standardizes gene expression while taking into account technical covariates such as sequencing depth and cell-specific variations. We used an anchor-based integration method to correct batch effects by identifying shared features (anchors) between different batches to align the datasets. The integration quality was verified through UMAP (Uniform Manifold Approximation and Projection) visualization and marker gene identification to ensure that the downstream analysis results have biological significance. UMAP was combined with t-SNE (t-Distributed Stochastic Neighbor Embedding) for dimensionality reduction analysis. To precisely define cell types, we first used SingleR for cell type annotation, and then further confirmed the cell

types based on the currently recognized characteristic molecules of immune cells [27]. We used the FindAllMarkers function in Seurat to explore genes highly expressed in certain subgroups and selected significantly highly expressed genes with|Log2FC > 0.1| and adjusted *p*-value < 0.05 (the Log2FC values in scRNA-seq are usually low, so the threshold was set at 0.1).

Transcriptome differential expression analysis

For the analysis of bulk RNA-seq data, we employed two R packages, DESeq2 and limma, which are standard tools for differential gene expression analysis [28, 29]. DESeq2 was used to normalize the read counts across samples, considering the differences in sequencing depth and sample composition. "Limma" package was then applied to identify DEGs between SLE patients and healthy controls. DEGs were defined as those with a|Log2FC| > 0.5 and an adjusted *p*-value < 0.05, ensuring that only biologically meaningful changes were considered.

Microarray datasets analysis

Microarray datasets from multiple platforms were processed using standard preprocessing pipelines. Raw microarray data were background-corrected, normalized using the Robust Multi-array Average (RMA) method, and log2-transformed to ensure comparability across datasets. RNA-seq datasets were analyzed using DESeq2 and limma for differential gene expression analysis, with normalization performed to account for sequencing depth and compositional differences. To harmonize RNA-seq and microarray datasets, we employed the RRA method, which ranks genes by significance within each dataset and aggregates these ranks across all datasets. This platform-independent, rank-based approach mitigates biases arising from differences in gene coverage and measurement techniques. Differentially expressed genes (DEGs) were identified based on aggregated ranks, with a cutoff for statistical significance set at an adjusted *p*-value < 0.05 and a|Log2FC| > 0.5.

Protein interaction analysis

To explore the functional interactions between DEGs, we conducted protein-protein interaction (PPI) analysis using the STRING database (version 11.0). This database provides a comprehensive view of known and predicted protein interactions, which is crucial for understanding the complex networks that underlie biological processes. The PPI analysis allowed us to visualize interaction networks and identify key nodes that may play central roles in SLE pathogenesis. These networks were analyzed and visualized using Cytoscape, an open-source software platform for network visualization and analysis. By identifying central nodes and pathways, we could pinpoint potential therapeutic targets and gain insights into the molecular mechanisms driving SLE.

Gene set enrichment analysis

In order to validate the cell subset signature gene sets we have established, we employed two single-sample gene set enrichment analysis approaches to examine whether the enrichment degree of the signature gene set in the relevant cell subset is more pronounced than that in other cell subsets.

The two main methods we utilized were the AUCell tool within the SCENIC single-cell analysis workflow and the Gene Set Variation Analysis (GSVA). The AUCell tool in the SCENIC single-cell analysis workflow computes the enrichment degree of a specific functional gene set in each cell. A higher enrichment value indicates a greater degree of enrichment [30]. GSVA is a commonly employed bioinformatics tool for calculating the enrichment degree of a specific gene set in a single sample based on transcriptome expression profile data, and it can also be utilized to analyze the enrichment or activation degree of the signature gene set in a single cell [31]. We visualized and compared the enrichment degree of single-cell marker gene sets using analytical methods in Seurat.

We performed Gene Set Enrichment Analysis (GSEA) [32] to determine the predefined sets of genes, particularly those related to immune pathways and IFN signaling, were significantly enriched in SLE patients compared to controls. GSEA is a computational method that assesses whether a set of genes shows statistically significant, concordant differences between two biological states.

Gene sets with a normalized enrichment score (NES) greater than 1 in absolute value and a false discovery rate (FDR) q-value less than 0.25 were considered significantly enriched. This analysis provided insights into the biological processes that are dysregulated in SLE and highlighted pathways that may contribute to disease progression.

Molecular pathway enrichment analysis

We conducted functional annotation of DEGs using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) to gain a deeper understanding of the molecular pathways involved in SLE. DAVID is a comprehensive bioinformatics resource that facilitates the functional interpretation of large-scale genomic data by identifying overrepresented biological processes and pathways. The analysis focused on Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [33]. GO terms provided insights into the biological processes, cellular components, and molecular functions associated with the DEGs, while

KEGG pathways highlighted the metabolic and signaling pathways that are dysregulated in SLE.

Mass cytometry data analysis

We downloaded the mass cytometry data from healthy individuals (FR-FCM-Z2XC) [34] obtained from FlowRepository (flowrepository.org/), a publicly available database for flow cytometry data. We used FlowJo software for initial gating of mononuclear phagocytes ($CD45^+CD3^-CD19^-$) to isolate the monocyte populations of interest. The data were then further analyzed using R, employing the t-SNE (t-distributed stochastic neighbor embedding) algorithm for dimensionality reduction and visualization.

Flow cytometry

Peripheral blood collected from SLE patients and healthy controls was resuspended in phosphate buffer solution (PBS) as a single cell suspension at a density of 1×10^7 cells/ml. Cells were stained with the combinations of CD14-FITC (Biolegend#367116) and CD169 (SIGLEC1)-PE (Biolegend#346004) mAbs against cell surface markers in PBS. After fixing and permeabilizing with Fixation/Permeabilization solution (ebioscience#00512343,00522356) and Permeabilization Buffer (ebioscience#00833356), cells were blocked by Human Fc block (Biolegend#422302) and then stained for nuclear transcription factors with anti-IRF7-Alexa Fluor 647 (ebioscience#51537542). Results were acquired using Cytomic FC500 and analyzed using FlowJo. Peripheral blood of SLE patients was obtained from the Department of Rheumatology and Immunology, The First Hospital of Xiamen University. Our work was performed with the consent of the patients, and the informed consent form was signed and approved by the ethics committee of our hospital, 2024Scientific Research Ethics Approval (Ethic No.218).

Statistical analysis

All statistical analyses were conducted using R software (version 3.6.1) and GraphPad Prism (version 8). Differences between groups were assessed using appropriate statistical tests, including t-tests for continuous variables and chi-square tests for categorical variables. The Mann-Whitney U test or Kruskal-Wallis H test is used to analyze the differences between groups of samples with unequal variances. A *p*-value of less than 0.05 was considered statistically significant, indicating a meaningful difference between the groups. For correlation analyses, Pearson or Spearman correlation coefficients were calculated depending on the distribution of the data.

Results

Interferon-driven transcriptomic reprogramming in SLE monocytes

Our analysis began with a comprehensive examination of monocyte transcriptomes using both microarray and RNA-seq data from 10 groups of SLE patients and matched healthy controls (Table 1). The integration of these datasets using the RRA method revealed a distinct set of DEGs that were significantly altered in monocytes from SLE patients. Notably, we identified an upregulation of key ISGs, including IFI27, RSAD2, IFIT3, USP18, and DDX58 (Fig. 1a). In total, 72 genes were significantly upregulated in SLE monocytes (Supplementary Table 4) forming what we termed the SLERRAsignature. To further explore the functional implications of these DEGs, we conducted PPI analysis using the STRING database. The analysis revealed a complex network of interactions among the upregulated genes, particularly those involved in the IFN signaling pathway (Fig. 1b and c). This network suggests that the aberrant expression of these genes may play a central role in the pathogenesis of SLE, particularly in the amplification of inflammatory responses.

GSEA analysis of the SLERRAsignature indicated a significant enrichment of pathways related to viral infection and IFN-I responses in SLE monocytes compared to healthy controls (Fig. 1d and e). These findings highlight the critical role of IFN signaling in the transcriptional reprogramming of monocytes in SLE, which may contribute to the systemic inflammation and autoimmunity observed in these patients.

Then, we analyzed the diagnostic role of the SLERRAsignature for SLE using transcriptomic data. GSEA analysis suggested that the SLERRAsignature in E-MTAB-2713 was significantly enriched in SLE patients (Fig. 1f). The GSVA enrichment score for the SLERRAsignature was significantly higher in the SLE group than the control group (Fig. 1g). Receiver operating characteristic curve (ROC) analysis showed that the GSVA enrichment score of SLERRAsignature was advantageous for the diagnosis of SLE (AUC: 0.93, 95% CI 0.88–0.97; Fig. 1h). This suggests that the SLERRAsignature could be a valuable tool for identifying SLE patients based on monocyte transcriptomes.

scRNA-seq unveils subset alterations in SLE mononuclear phagocytes

To dissect the alterations in the composition of mononuclear phagocytes within the peripheral blood of SLE patients, following rigorous quality control and data integration, we analyzed mononuclear phagocyte subsets from scRNA-seq datasets (Supplementary Table 1). The datasets included four distinct groups: healthy control, inactive SLE (SLEDAI ≤ 10), severe SLE (SLEDAI > 10) [35, 36], and flare (recurrence). At first, we normalized

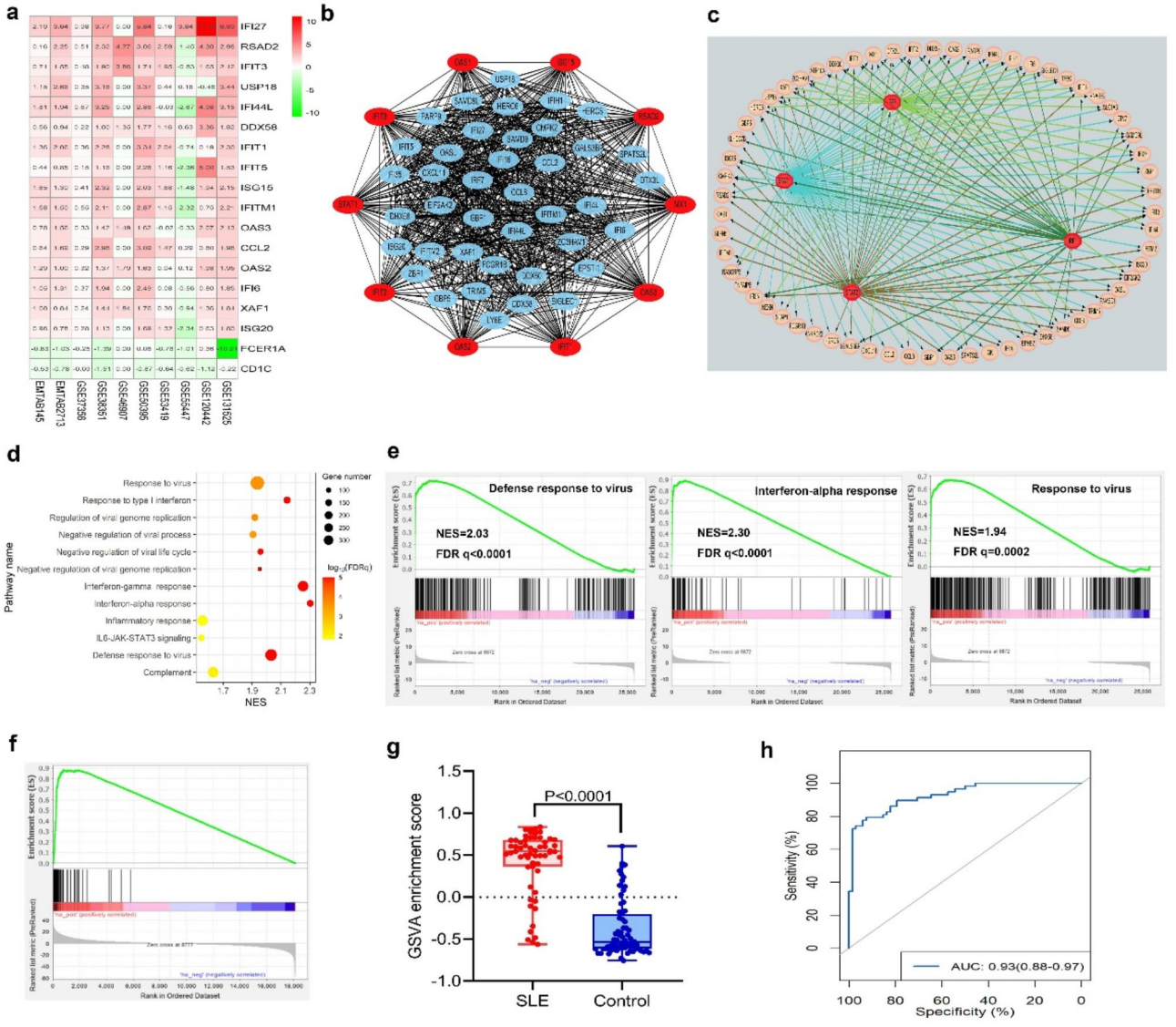

Fig. 1 RRA analysis of the key genes and functional pathways from SLE monocytes, and verified the transcriptional signature gene set of SLE monocytes. (**a**) Identification of key differentially expressed genes through RRA analysis. (**b**) STRING network analysis illustrating the protein-protein interaction (PPI) network among these differentially expressed genes. (**c**) The network regulatory relationship diagram showed significantly enriched TFs and their regulatory targets. Red represents TFs and brown represents regulatory genes. (**d**) GSEA analysis based on the differentially expressed gene list from the RRA analysis. (**e**) GESA enrichment map of key signaling pathways. (**f**) GSEA analysis indicates a significant enrichment of the SLE monocyte transcriptional signature gene set in SLE patients. (**g**) The GSVA enrichment scores of the SLE monocyte transcriptional signature gene set compared with the control. (**h**) ROC analysis reveals that the GSVA enrichment scores of the SLE monocyte transcriptional signature gene set

and integrated data from these 20 samples, resulting in a dataset comprising 29,409 mononuclear phagocytes. This analysis identified nine $CD14^+$ monocyte subsets (CD14Mono1-9), two $CD16^+$ subsets (CD16Mono1-2) and two DC subsets, including conventional dendritic cells (cDCs) and plasmacytoid dendritic cell (pDC) (Fig. 2a and b).

Composition ratio analysis was conducted after randomly selecting 5500 mononuclear phagocytes from all SLE groups and healthy controls (Fig. 2c). Subsequently we compared and analyzed the differences of the composition ratio in peripheral blood mononuclear phagocyte subsets among different groups (Fig. 2d and e). Our analysis revealed that, in comparison with healthy controls, SLE patients exhibited a higher frequency of CD14Mono4 and CD16Mono2, whereas the frequency of pDC in SLE patients was inclined to be lower. It is notable that there is a tendency to increase in the proportion of the CD14Mono8 subset in the flare group compared to controls (Fig. 2c-e), suggesting that CD14Mono8 monocytes may play a crucial role in disease exacerbation and could serve as a biomarker for predicting flares in SLE

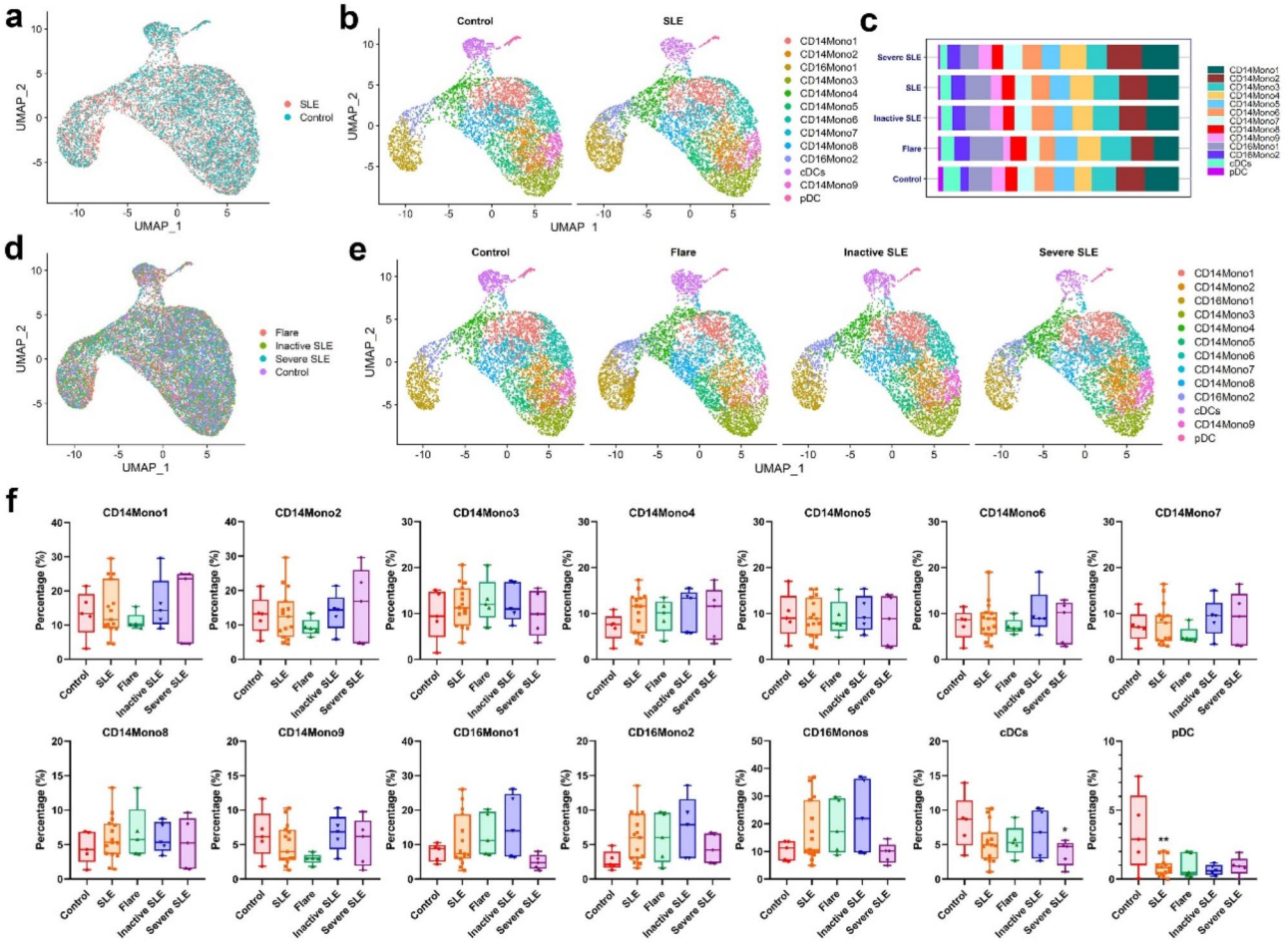

Fig. 2 Analysis of Peripheral Blood Mononuclear Cell Subsets in Systemic Lupus Erythematosus Patients. (**a**) UMAP dimensionality reduction showing the distribution of cells of SLE group and healthy control group. (**b**) UMAP dimensionality reduction comparing the distribution of different subsets of SLE group and healthy control group. (**c**) Bar chart analysis of the composition of mononuclear phagocyte subsets in the SLE group, healthy control group, and different SLE clinical subtypes. (**d**) UMAP dimensionality reduction showing the distribution of cells of the three SLE clinical subtypes and the healthy control group. (**e**) UMAP dimensionality reduction comparing the distribution of different subsets of the three SLE clinical subtypes and the healthy control group. (**f**) Analysis of the differences in the proportions of mononuclear phagocyte subset between the groups. *$p < 0.05$, **$p < 0.01$

patients. After individually calculating the proportion of each subset for each sample and conducting a statistical analysis between groups, it was found that the proportion of cDCs in patients with severe SLE was significantly lower than that in healthy control group ($p < 0.05$), and there were no statistically significant differences in the composition of other mononuclear phagocyte subsets between the groups ($p > 0.05$) (Fig. 2f).

Differential transcriptomic profiling highlights ISG activation in SLE monocytes

Next, we analyzed the differential gene expression of mononuclear phagocyte subsets and found that 13 subsets with upregulated DEGs in SLE patients were dominated by IFN-stimulated genes such as ISG15, IFITM3, LY6E, IFI6, IFI44L, EPSTI1, and STAT1 (Fig. 3a-c). GSEA of the CD14Mono8 subset indicated significant enrichment of IFN-related pathways, including the IFN-α response, IFN-γ response, and defense response to viral infection (Fig. 3d), the same situation was also observed in pDC (Fig. 3e). These findings underscore the central role of IFN signaling in the transcriptional reprogramming of monocytes in SLE. Compared to inactive SLE patients, the upregulated genes in all 13 mononuclear phagocyte subsets in severe SLE patients were predominantly ISGs, such as ISG15, IFITM3, IFI6, and IFI44L (Supplementary Fig. 2a-c). This suggests that the severity of SLE is closely linked to the activation of IFN-driven pathways, which may contribute to the progression and exacerbation of the disease.

SLERRAsignature as a marker for SLE clinical progression and ISG expression

Compared to healthy controls, the enrichment of the SLERRAsignature, established in the first part of this study, was significantly higher in various mononuclear

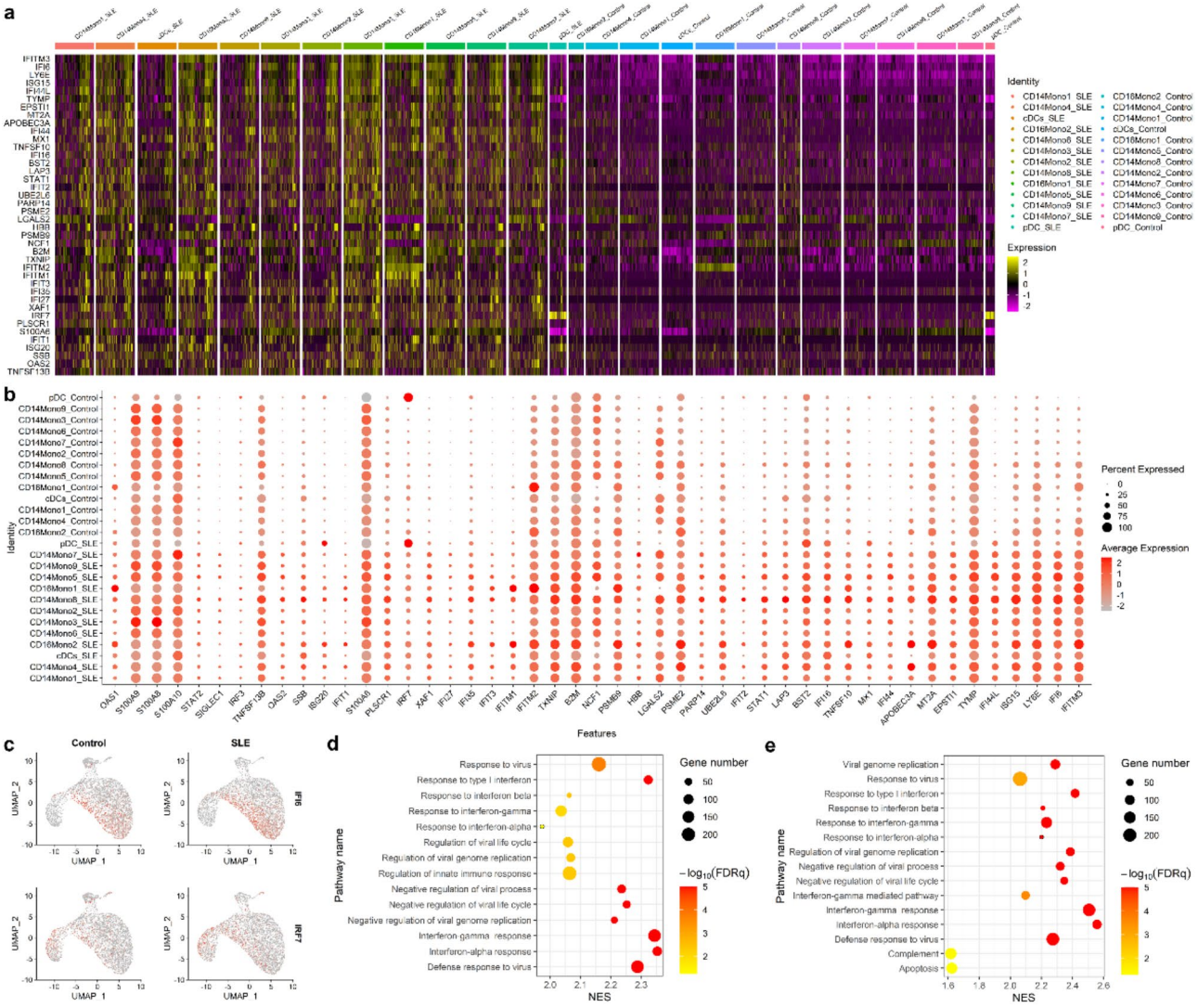

Fig. 3 Differential expression analysis of transcriptomes of monocyte subsets in SLE patients and healthy controls. (**a**) Heatmap showing the expression of selected key ISGs in mononuclear phagocyte subsets of SLE patients and healthy controls. (**b**) Dot plot comparing the expression of selected key ISGs in mononuclear phagocyte subsets of SLE patients and healthy controls. (**c**) Feature plot comparing the expression of IFI6 and IRF7 in mononuclear phagocytes of SLE patients and healthy controls. (**d**) GSEA analysis showing the significantly upregulated signaling pathways in CD14Mono8 cells of SLE patients. (**e**) GSEA analysis showing the significantly upregulated signaling pathways in pDC of SLE patients

phagocyte subsets of SLE patients with different clinical subtypes (Fig. 4a and b). Additionally, the enrichment of the SLERRAsignature in mononuclear phagocyte subsets of severe SLE patients was substantially more pronounced than that in those of inactive SLE patients (Fig. 4a and b). Furthermore, the expression levels of key ISGs were generally increased in mononuclear phagocyte subsets of SLE patients with different clinical subtypes compared to healthy controls, and the expression levels of key ISGs in severe SLE patients were significantly higher than those in inactive SLE patients across all mononuclear phagocyte subsets (Fig. 4b). Interestingly, it was also found that the SLERRAsignature was mainly enriched in the CD14Mono8 subset (Fig. 4b), and in this subset the expression level of the SLERRAsignature was correlated with the severity of the disease, indicating that this group of cells might have the potential to reflect the disease activity. However, further exploration of its characteristics is necessary.

High-quality scRNA-seq unveils the precise features of the subsets of peripheral blood mononuclear phagocytes

After a thorough analysis of the enrichment of SLERRAsignature, we need to analyze the gene characteristics of each monocyte subset more precisely to discover the characteristics of the cell subset enriched with SLERRAsignature. We got high-quality scRNA-seq data by rigorously screening (Supplementary Table 2) and finally

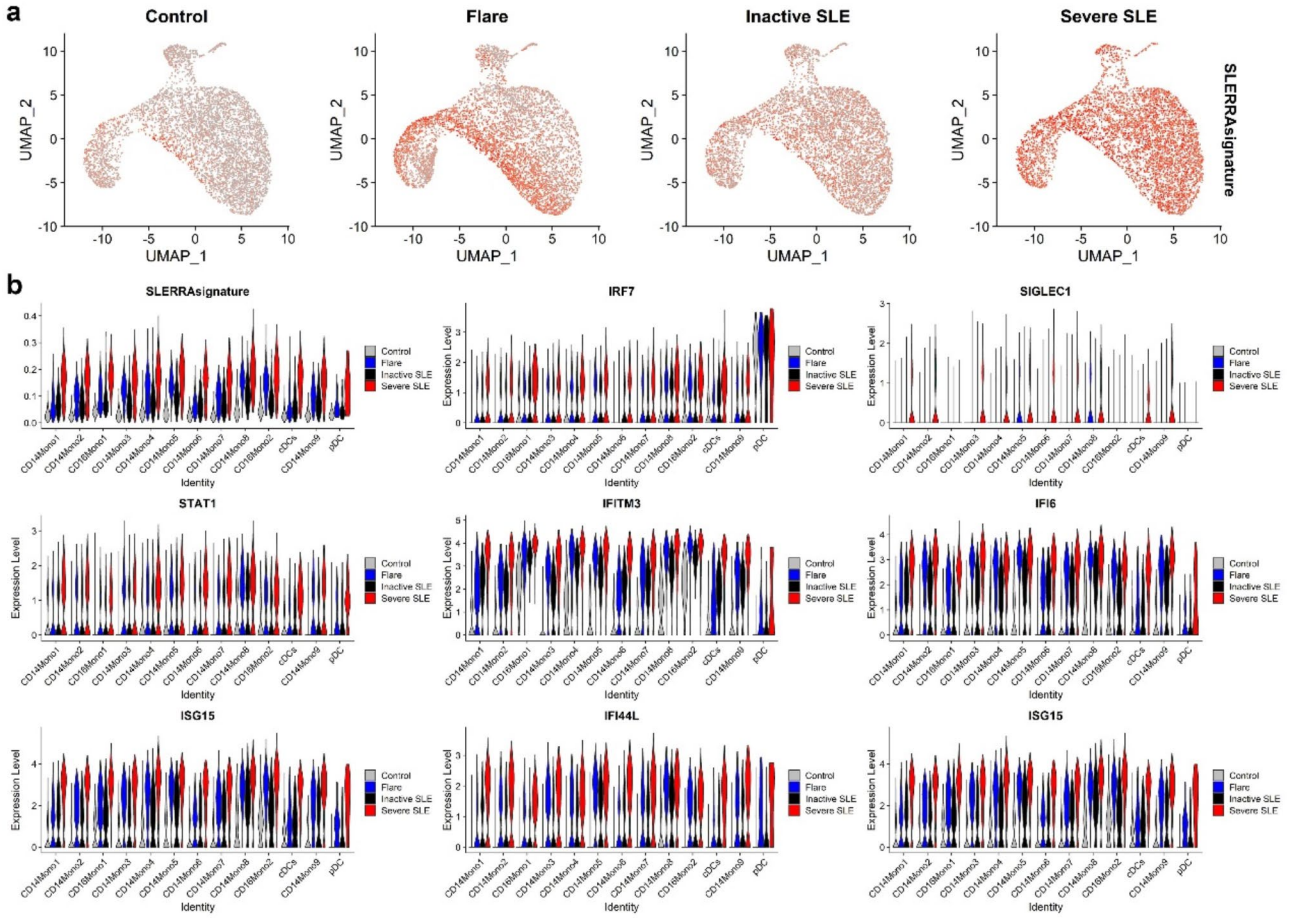

Fig. 4 SLERRAsignature and ISG expression in mononuclear phagocytes across different clinical stages of SLE. (**a**) Feature plot comparing the enrichment of the SLERRAsignature in mononuclear phagocytes across different clinical subtypes of SLE patients. (**b**) Violin plot comparing the changes in SLERRAsignature and key ISGs across mononuclear phagocyte subsets in different clinical subtypes of SLE patients

obtained a dataset of 25,557 mononuclear phagocytes, including eight $CD14^+$ monocyte subsets, two $CD16^+$ monocyte subsets, one pDC subset and one cDCs subset (Fig. 5a). The expression of common cell marker molecules in each mononuclear phagocyte population was shown in Fig. 5b and c. In addition, UMAP dimensionality reduction clustering plots of mononuclear phagocytes from each sample were shown (Fig. 5d). Among them, the CD14Mono7 subset highly expressed SIGLEC1 (CD169) and IRF7, thus we defined it as $SIGLEC1^+IRF7^+$ monocytes. Similar to the CD14Mono8 subset in the previous section, it was a subset of mononuclear phagocytes that highly express STAT1, IRF7 and other IFN pathway-related genes.

Identification of $SIGLEC1^+IRF7^+$ monocytes in healthy individuals

Furthermore, $SIGLEC1^+IRF7^+$ monocytes (CD14Mono7) were also present in the peripheral blood of healthy individuals (Fig. 6a and b). This subset of cells was not previously identified in peripheral blood mononuclear phagocytes scRNA-seq studies, making this the first study to discover this subset in the peripheral blood of healthy individuals. Compared to other monocytes, this subset in healthy individuals exhibited higher expressions of molecules such as SIGLEC1, IRF7, and STAT1 (Fig. 6c and d). Mass cytometry data from the peripheral blood of healthy individuals also confirmed the presence of a certain proportion of $SIGLEC1^+$ monocytes. In healthy individuals, $SIGLEC1^+$ mononuclear phagocytes were primarily composed of $CD14^+$ monocytes, with smaller proportions of $CD16^+$ monocytes and cDCs (Fig. 6e).

Establishing and validating the CD14Mono7NoISGs gene set for monocyte subset analysis

For the purpose of identifying the characteristic genes of various cell subsets, we adopted the SCTransform integration method in Seurat for the analysis of RNA-seq data from six high-quality PBMCs (GSE140228, GSE146771, GSM3892570, GSM3892571, TenX10KV3, and TenXSC3V3). Subsequently, the signature gene sets

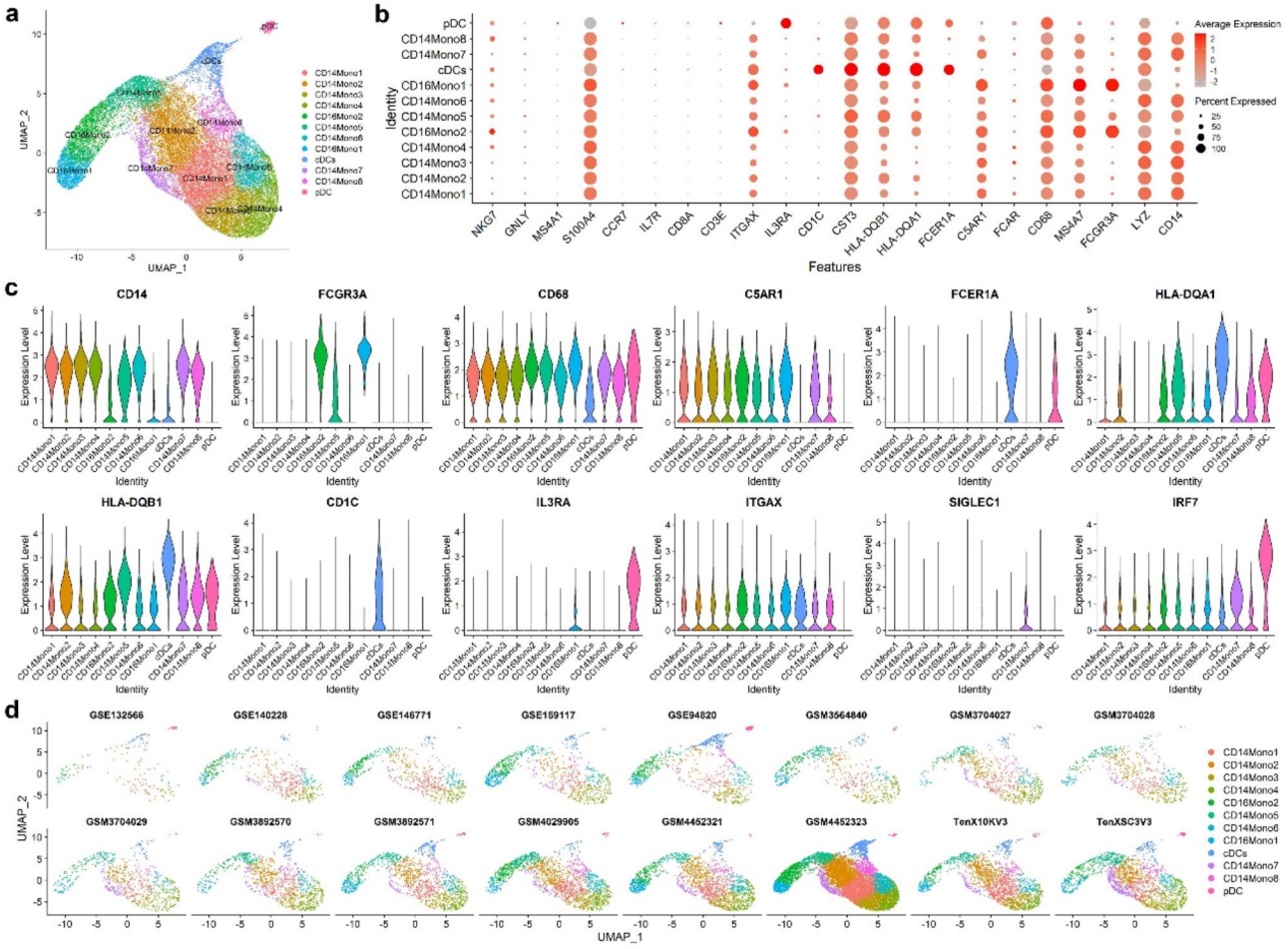

Fig. 5 RNA-seq analysis of peripheral blood mononuclear cells and DCs data. (**a**) UMAP dimensionality reduction grouping of 25,557 mononuclear phagocytes was performed. (**b**) Comparative analysis of common cell markers in a dot-plot manner. The expression of the molecules in each mononuclear phagocyte population was recorded. (**c**) Violin plot for comparative analysis of common cell marker molecules in each single expression of nuclear phagocyte clusters. (**d**) UMAP dimensionality reduction clustering plot of mononuclear phagocytes for each sample

of various immune cells were identified by FindAllMarkers, and the top 50 were listed (Supplementary Table 5).

Additionally, we removed the co-expressed ISGs in monocytes and non-monocytes (such as B cells, T cells, and NK cells) to create a gene set named CD14Mono7NoISGs. This gene set reduces the interference of non-monocyte-expressed ISGs to the whole blood transcriptome data and can efficiently analyze the changes of CD14Mono7 cells in whole blood cells s or PBMCs transcriptome data (Supplementary Table 5).

Two single-sample gene set enrichment analysis tools (AUCell and GSVA) were used to verify the signature gene sets of the mononuclear phagocyte subsets. In AUCell analysis with data from GSM4452323, the results indicated that the signature gene sets of CD14Mono7, CD16Mono, cDCs, and pDC were more abundant in the corresponding subsets, effectively representing each subset (Fig. 7a and b). The CD14Mono7NoISGs gene set was significantly more enriched in CD14Mono7 than in other subsets, suggesting it could be a representative of CD14Mono7 (Fig. 7b). GSVA analysis using GSE140228 data also supported that the above signature gene sets effectively represented the corresponding mononuclear phagocyte subsets (Supplementary Fig. 3).

Correlation of CD14Mono7 signature with SLEDAI

Based on the subsets signature gene sets established above, we analyzed the clinical relevance of CD14Mono7 and CD14Mono7NoISGs signatures. Firstly, the RRA analysis results of PBMCs from the first part datasets (Table 1) were extracted and analyzed by GSEA method. Next, the transcriptome data of whole blood cells from SLE patients (Supplementary Table 3) were integrated by RRA method for GSEA analysis. The CD14Mono7 and CD14Mono7NoISGs signature gene sets were significantly enriched in the PBMCs (Fig. 8a and b) and whole blood cells (Fig. 8c and d) of SLE patients, suggesting a possible increase in the number of CD14Mono7 subset

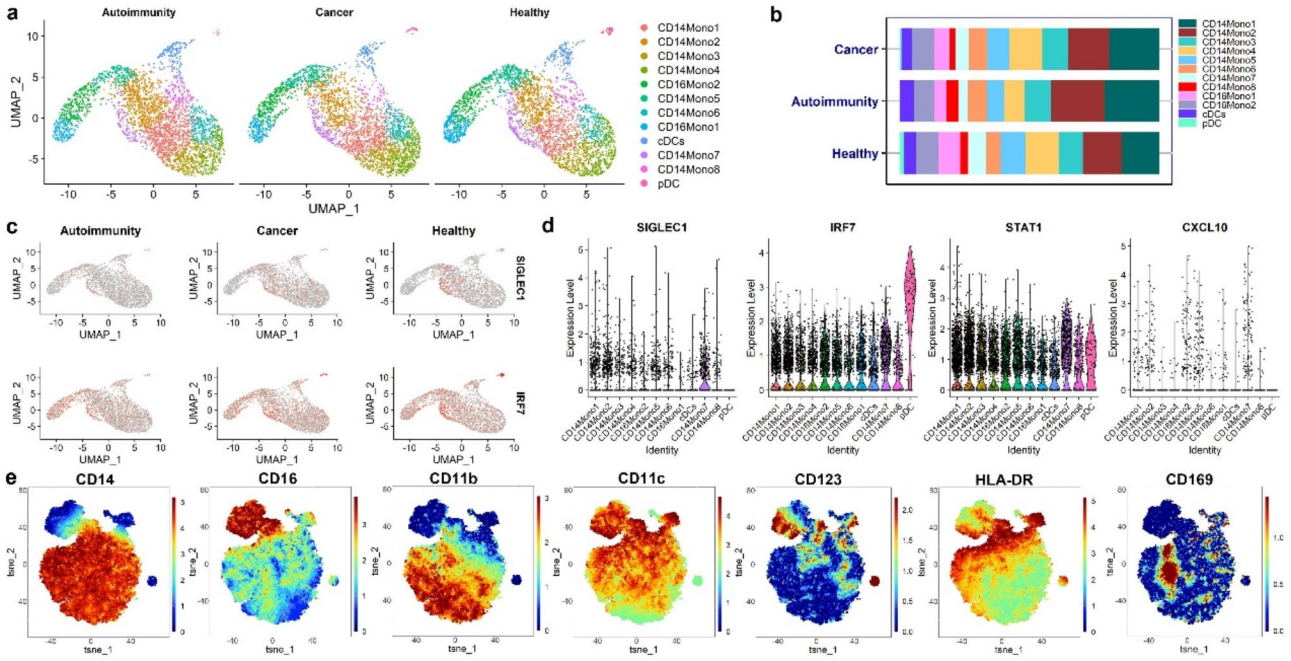

Fig. 6 SIGLEC1^{+}IRF7^{+} monocytes (CD14Mono7) in peripheral blood of healthy people. (**a**) UMAP dimensionality reduction clustering of mononuclear phagocytes from samples across different groups. (**b**) Comparison of the composition proportions of mononuclear phagocytes in samples from different groups. (**c**) In the peripheral blood of healthy individuals, the CD14Mono7 subset shows higher expression of SIGLEC1 and IRF7 compared to other monocytes. (**d**) In the peripheral blood of healthy individuals, the CD14Mono7 subset shows higher expression of SIGLEC1, IRF7, STAT1, and other molecules compared to other monocytes. (**e**) Mass cytometry data confirms the presence of a certain proportion of SIGLEC1^{+} monocytes in the peripheral blood of healthy individuals

cells both in their PBMCs and whole blood cells. Additionally, the CD16Mono signature gene set was enriched in the whole blood cells of SLE patients (Fig. 8c and d), indicating a potential increase in CD16^{+} monocytes. The enrichment of the pDC signature gene set was significantly downregulated in the whole blood cells of SLE patients (Fig. 8c and d), suggesting a possible decrease in pDC, which is consistent with the scRNA-seq analysis results of SLE mononuclear phagocytes from the previous part of the study.

We used GSE88884 data to analyze the correlation between the enrichment scores of the mononuclear phagocyte subsets signatures in the whole blood cells of SLE patients and clinical parameters, including the SLEDAI. As shown in Fig. 8e, both the enrichment scores of CD14Mono7 and CD14Mono7NoISGs signatures had a significantly positive correlation with SLEDAI, suggesting that the expansion of the CD14Mono7 subset may be associated with high disease activity. Additionally, the enrichment scores of the CD14Mono7NoISGs signatures were negatively correlated with the levels of C3 and C4, and positively correlated with anti-dsDNA titers (Fig. 8e), further supporting the pathogenic role of this monocyte subset in SLE.

Elevated CD14^{+}SIGLEC1^{+}IRF7^{+} monocytes distinguish SLE patients from healthy controls and reflect disease activity

The correlation between CD14^{+}SIGLEC1^{+}IRF7^{+} monocytes enrichment and clinical indicators emphasizes its importance as a potential biomarker for SLE disease activity. Thereby, we used flow cytometry to examine the proportion of CD14^{+}SIGLEC1^{+} and CD14^{+}SIGLEC1^{+}IRF7^{+} monocytes (CD14Mono7) in the peripheral blood of SLE patients ($n = 31$) and healthy controls ($n = 10$). The Demographic data and clinical characteristics of all samples analyzed in our study are exhibited in Table 2. We categorized patients into two groups based on their SLEDAI scores: SLEDAI > 10 ($n = 18$) and SLEDAI ≤ 10 ($n = 13$) (the classification criteria were the same as the SLE scRNA-seq analysis described above).

In Fig. 9a, the flow cytometry gating strategy for the healthy controls and two SLE patient groups is shown, which identifies the CD14^{+}CD169^{+} cells population. In Fig. 9b, on the basis of gating the CD14^{+} cells, the CD169^{+}IRF7^{+} monocytes population is further defining. The results demonstrated that the proportion of CD14^{+}CD169^{+} monocytes in SLE patients was significantly higher compared to healthy controls (Fig. 9c). Within the SLE group, while no significant difference was observed between the two groups with different disease activity, both groups exhibited significant differences

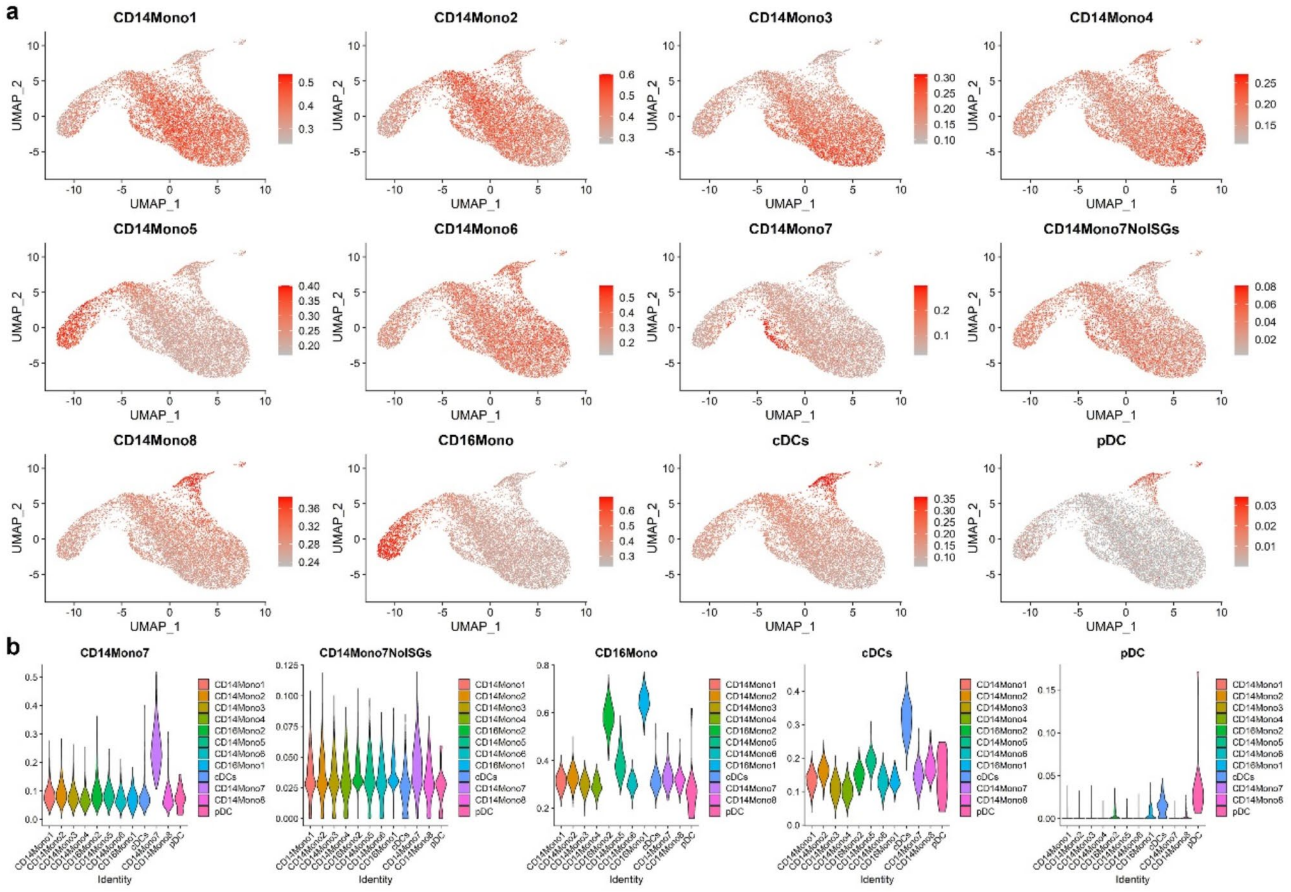

Fig. 7 AUCell in the SCENIC single-cell analysis pipeline was used to verify the signature gene sets of mononuclear phagocyte subsets. (**a**) The enrichment of signature gene sets of mononuclear phagocyte subsets in each cell population were compared and analyzed by feature map. (**b**) The enrichment of mononuclear phagocyte subsets signature gene sets in each cell population were compared and analyzed by violin plot

relative to healthy controls (Fig. 9d). Figure 9e shows that the proportion of $CD14^+CD169^+IRF7^+$ monocytes in SLE patients was markedly elevated compared to healthy controls. Intriguingly, both groups of SLE patients formed significant differences from healthy controls, and there were also significant differences between the two groups of patients. The more severe the disease, the higher the proportion of this population of cells (Fig. 9f). Additionally, we examined the proportion of $CD14^+CD169^+IRF7^+$ monocytes within the $CD14^+CD169^+$ population (Supplementary Fig. 4a), observing no significant difference between SLE and healthy controls or between the two groups of patients ($p > 0.05$) (Supplementary Fig. 4b and 4c). Collectively, our flow cytometry analysis suggests that the elevation of $CD14^+CD169^+IRF7^+$ monocytes may be a potential indicator for distinguishing healthy controls from SLE patients. Furthermore, the detection of $CD14^+CD169^+IRF7^+$ monocytes may serve as a valuable tool for assessing SLE severity.

Discussion

The IFN-I pathway plays a central role in the pathogenesis of SLE [10, 37], by activating downstream signaling pathways and transcription factors through Janus kinase (JAK) [4, 8] and contributing to the breakdown of immune tolerance, chronic inflammation, and autoimmunity. Abnormal activation of the IFN pathway might result in promoting the presentation of self-antigens [38–42], augmenting Th17 cells [43], concurrently inhibiting the function of Treg cells [43], and sustaining the long-term survival of autoreactive B cells [44], thereby disrupting immune tolerance in patients with SLE.

It is widely recognized that IFN-I in peripheral blood is primarily produced by pDC [20, 45, 46] and has been shown to play a role in the pathogenesis of SLE [20]. It has also been shown that although the pDC is responsible for the majority of IFN-α production in the peripheral blood of healthy people, it accounts for only 57% of the IFN-α production in the peripheral blood of SLE [47]. Recent research indicates that certain monocytes are also involved in IFN-I production and are regulated by IFN-I, contributing to a crucial positive feedback loop of IFN-I

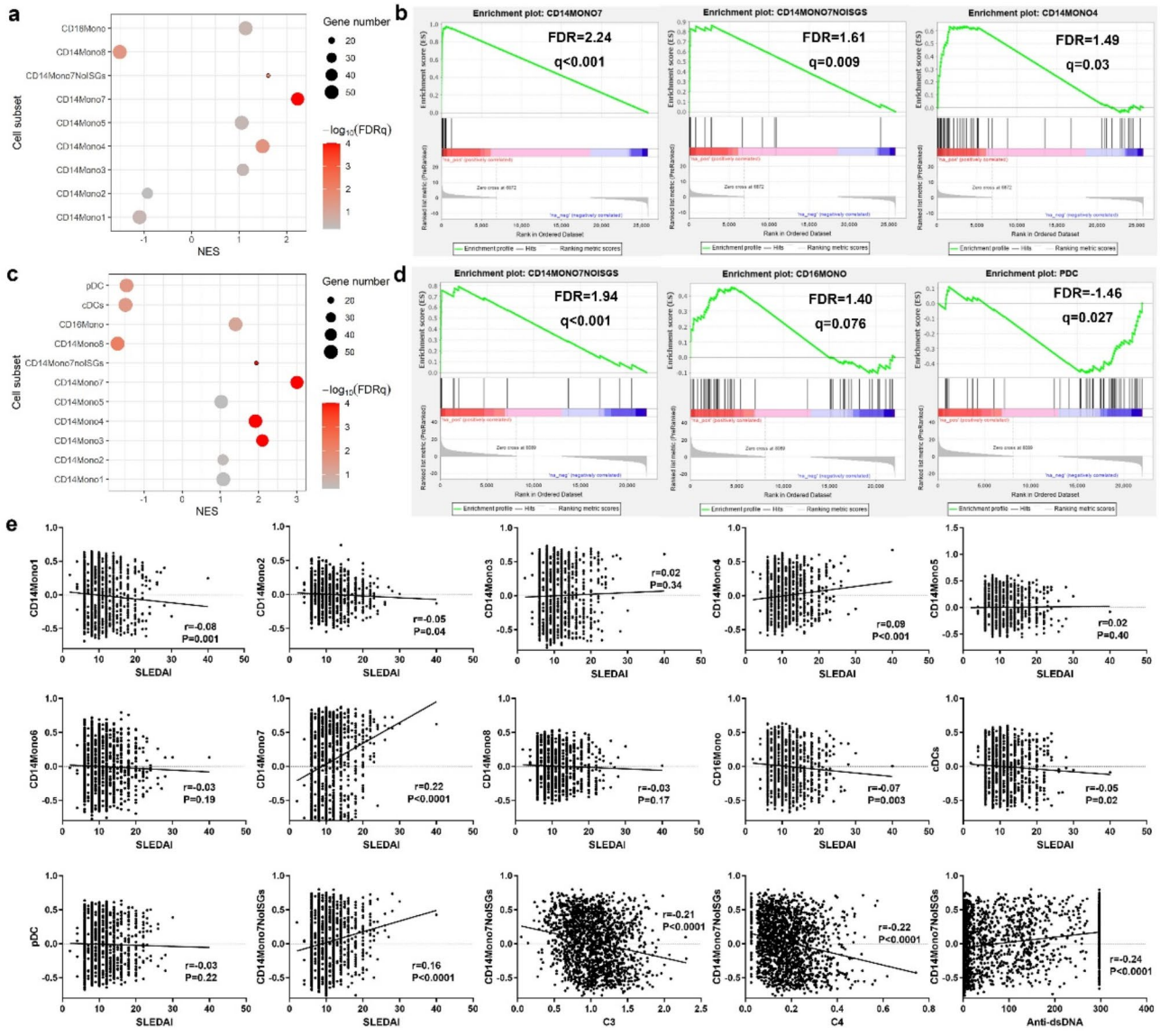

Fig. 8 Enrichment and correlation of CD14Mono7 signature gene sets in SLE. (**a**) Bubble plot showing the enrichment of mononuclear phagocyte subset signature gene sets in monocytes of SLE patients. (**b**) GSEA enrichment plot of key mononuclear phagocyte subset signature gene sets in monocytes of SLE patients. (**c**) Bubble plot showing the enrichment of mononuclear phagocyte subset signature gene sets in the peripheral blood of SLE patients. (**d**) GSEA enrichment plot of key mononuclear phagocyte subset signature gene sets in the peripheral blood of SLE patients. (**e**) Correlation between the enrichment values of mononuclear phagocyte subset signature gene sets in whole blood of SLE patients and clinical indicators

[8]. Furthermore, abnormalities in the number or function of monocytes have been found to promote the onset and progression of SLE [48, 49]. Nevertheless, these studies failed to provide a thorough classification of the pathogenic monocyte subsets and did not examine their biological markers. Our study aims to identify a specific subset of monocytes implicated in the pathogenesis of SLE, uncover its characteristic genetic markers, and analyze the clinical relevance of this subset of monocytes with the goal of providing new insights for future diagnosis and treatment strategies for SLE.

Our study found that key ISGs such as IFI27 and RSAD2 were significantly up-regulated in monocytes of SLE patients, forming a SLERRAsignature, which was significantly enriched in viral infection and IFN-I response pathways, and its GSVA enrichment score was conducive to SLE diagnosis. However, due to the lack of clinical data, it could not be verified by COX regression. In addition, the original data of GSVA analysis contained information on other autoimmune diseases [50], and the overlap of IFN characteristics affected the diagnostic specificity. Although SLE is different from other diseases, the high overlap suggests that multi-disease cohort

Table 2 Demographic data and clinical characteristics of subjects in the study

Characteristics	Patients with SLE ($n=31$)	HCs ($n=10$)	*P*-value
Age (Mean ± SD) (years)	32.84 ± 14.14	31.60 ± 5.52	0.70
Sex (% Female)	0.87	0.90	0.92
Height (cm)	160.23 ± 7.57	164.00 ± 5.14	0.42
Weight (kg)	55.31 ± 10.45	58.00 ± 5.80	0.33
Disease Activity (Mean SLEDAI ± SD)	9.68 ± 7.08	/	/
SLEDAI ≤ 10 (%)	22.00 (70.97%)	/	/
SLEDAI > 10 (%)	9.00 (29.03%)	/	/
C3 (Mean ± SD)	0.71 ± 0.33	/	/
C4 (Mean ± SD)	0.14 ± 0.09	/	/
ANA Positive (%)	1	/	/
Anti-dsDNA Antibody Positive (%)	0.55	/	/
Anti-Sm Antibody Positive (%)	0.19	/	/
Anti-Nucleosome Antibody Positive (%)	0.55	/	/

analysis is needed to clarify the specificity of IFN characteristics for SLE.

IFN response can reflect disease activity and severity [51, 52]. However, some studies have indicated that the degree of IFN-I response is not associated with long-term disease activity in individual SLE patients. This literature suggests potential bias when using the expression of IFN-I signature genes in whole blood cells or PBMCs as lupus disease activity biomarkers [53]. To address this limitation, the authors propose utilizing IFN response indices of specific cell subsets to enhance clinical assessment of IFN biomarkers. In this study, we established an SLE monocyte signature gene set based on transcriptome data from PBMCs of SLE patients for diagnosing and evaluating SLE. Additionally, we have established a distinct gene set for various monocyte subgroups through the analysis of SLE monocyte scRNA-seq. In the study, we not only strived to obtain as many sample types and quantities as possible, but also took into account our requirements for high-quality scRNA-seq data. Therefore, strict data screening was necessary. Ultimately, datasets encompassing cancer patients, those with drug-reaction with eosinophilia and systemic symptoms (DRESS), SLE, rheumatoid arthritis (RA) patients, and healthy controls were incorporated into the analysis. Despite potential limitations in the variety and number of these datasets, the high-quality nature of the samples ensured the reliability and robustness of the analysis outcomes. Going forward, future research endeavors should prioritize the collection of a larger volume of high-quality data, expansion of the sample size, and refinement of data processing methodologies.

We have identified a novel subset of monocytes, known as SIGLEC1$^+$IRF7$^+$ monocytes, through analysis of scRNA-seq and mass spectrometry of peripheral blood monocytes. Previous studies generally consider the expression level of SIGLEC1$^+$ in monocytes as an indicator of IFN-I activation, but do not classify SIGLEC1 monocytes as a distinct subset with specific functionality. The previous scRNA-seq analysis literature did not exclude the SIGLEC1$^+$IRF7$^+$ monocyte subset due to limited sample size and relatively low gene expression per cell [54, 55]. By augmenting the monocyte population in scRNA-seq analysis and selectively retaining only those with a substantial number of expressed genes, we enhanced the data quality of scRNA-seq analysis, thereby bolstering the efficacy of cell clustering and elevating the precision of data analysis. The identification of the SIGLEC1$^+$IRF7$^+$ monocyte subset in healthy individuals through scRNA-seq analysis signifies notable progress, as it provides the first confirmation of its existence and defines its transcriptomic signature.

SIGLEC1$^+$IRF7$^+$ monocytes exhibit a significantly elevated expression of the transcription factor IRF7, which plays a pivotal role in regulating the expression level of IFN-α [56–58]. Therefore, we postulated that SIGLEC1$^+$IRF7$^+$ monocytes may be the major monocyte subset that regulates the secretion of cytokines such as IFN-α. SIGLEC1 is a downstream target of IFN induction and is involved in immune complex clearance and antigen presentation by mononuclear cells [59]. In addition, the expression of SIGLEC1 can promote monocytes to secrete other cytokines such as IL-6 and TNF-α in response to IFN signals [60, 61] and regulate the activation ability of T cells [62]. We hypothesize that CD14Mono7 cells present in the peripheral blood of healthy individuals may play a crucial role in maintaining immune homeostasis. Further research is warranted to elucidate their specific functions.

Several studies have utilized scRNA-seq technology to investigate the pathogenesis of SLE and LN, identifying key immune cells and functional molecules involved in the disease process [21, 63, 64]. Nehar-Belaid et al. conducted scRNA-seq analysis of PBMCs from patients with SLE and observed significantly elevated expression levels of ISGs compared to healthy controls [21]. Furthermore, ISGs were found to be predominantly expressed in immune cell subsets including pDC, cDCs, CD4$^+$ T cells, CD8$^+$ T cells, NK cells, B cells, and a subset of monocytes. The data analyzed in our study were partly obtained from Nehar-Belaid et al. However, our study mainly analyzed monocytes and DCs, and the results of scRNA-seq analysis on monocytes in our study are significantly different from the conclusions of Nehar-Belaid et al. They suggested that a specific subset of monocytes in SLE patients exhibited high expression of ISGs, without

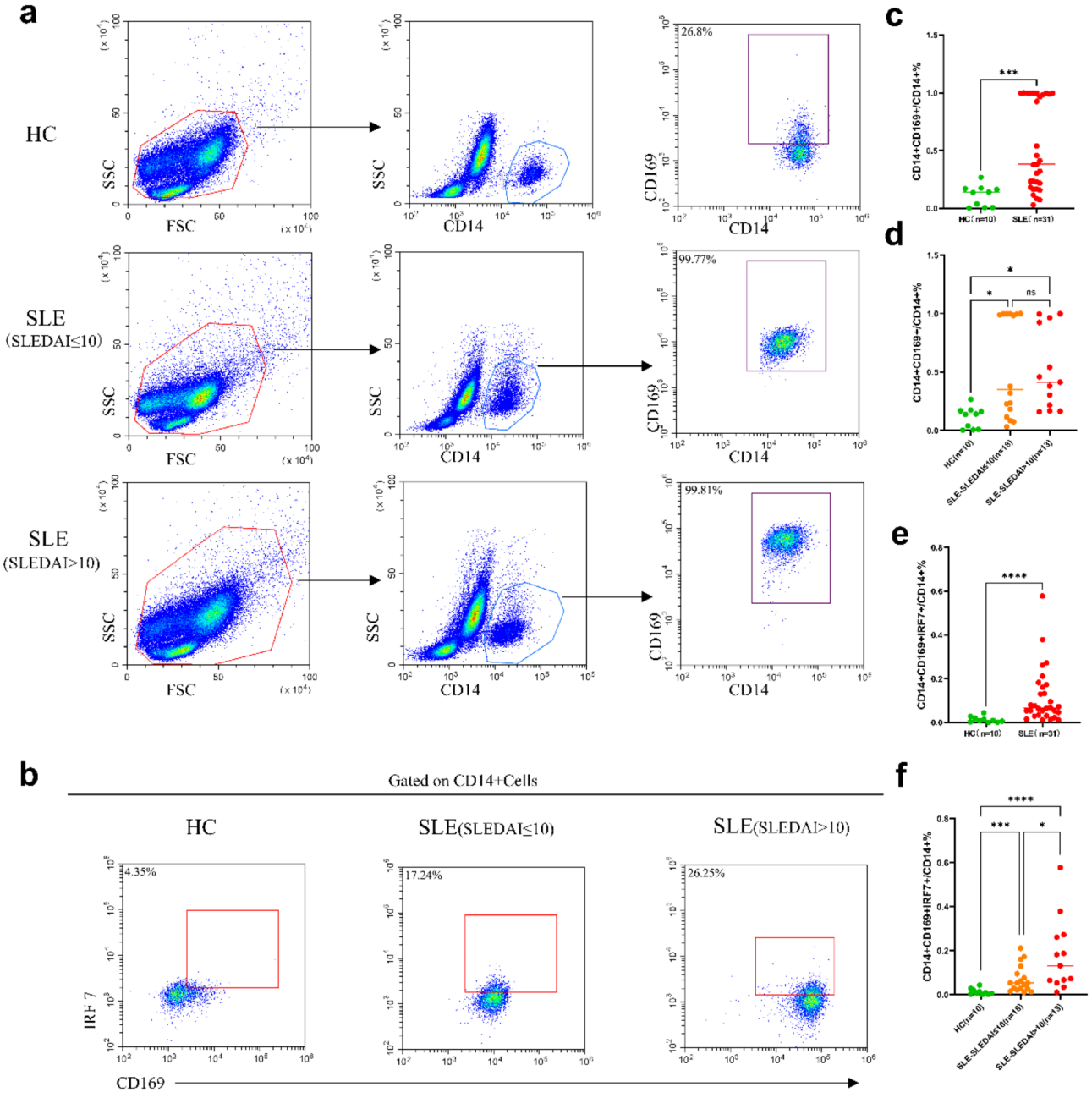

Fig. 9 Flow cytometry analysis of $CD14^+CD169^+IRF7^+$ monocytes in SLE patients. (**a**) Flow cytometric gating strategies for $CD14^+CD169^+$ monocytes in healthy controls and two groups of SLE patients. (**b**) The frequency of $CD14^+CD169^+IRF7^+$ cells under $CD14^+$ gating strategy in healthy controls and two groups of SLE patients. (**c**) The frequency of $CD14^+CD169^+/CD14^+$ monocytes in healthy controls ($n=10$) and SLE patients ($n=31$). (**d**) The frequency of $CD14^+CD169^+/CD14^+$ monocytes in healthy controls and SLE patients with SLEDAI > 10 ($n=18$) and SLEDAI ≤ 10 ($n=13$). (**e**) The frequency of $CD14^+CD169^+IRF7^+/CD14^+$ monocytes in healthy controls and SLE patients. (**f**) The frequency of $CD14^+CD169^+IRF7^+/CD14^+$ monocytes in healthy controls and two groups of SLE patients. (ns $p>0.05$, *$p<0.05$, ***$p<0.001$, ****$p<0.0001$)

further categorizing the monocytes for detailed analysis. By selecting high-quality scRNA-seq data and incorporating new SLE monocyte scRNA-seq data, the quality of data analysis and the accuracy of results were enhanced. Our scRNA-seq analysis revealed a novel monocyte subset, $SIGLEC1^+IRF7^+$ monocytes, and demonstrated that all monocytes, rather than only a subset, exhibited high expression of ISGs in SLE patients compared to healthy controls. This contrasts with the findings of Nehar-Belaid et al. Moreover, in our scRNA-seq results, we observed a decrement in peripheral blood pDC in SLE patients, which was in accordance with previous studies [65] and further solidified the reliability of our scRNA-seq analysis. Therefore, the scRNA-seq analysis of SLE monocytes

in this study further elucidates the role of monocytes and ISGs in the pathogenesis of SLE and offers insights into potential new targets for studying SLE treatment.

Furthermore, the enrichment value of the SIGLEC1$^+$IRF7$^+$ monocyte signature gene set exhibited a significant positive correlation with SLEDAI and anti-dsDNA, as well as a significant negative correlation with complement C3 and C4. This discovery suggests that the presence of SIGLEC1$^+$IRF7$^+$ monocytes in the peripheral blood of SLE patients is associated with disease activity, and individuals with higher levels of these monocytes may experience more severe symptoms. It implies that SIGLEC1$^+$IRF7$^+$ monocytes could potentially play a crucial role in the progression of SLE. Previous studies have indicated that the expression of SIGLEC1 on monocytes is associated with disease activity in SLE patients [66, 67], and it can be used to monitor the change of disease activity in SLE patients during follow-up [66, 68]. It has been demonstrated that the expression of SIGLEC1 in monocytes of SLE patients significantly decreased following effective treatment [69]. The expression of IRF7 is significantly associated with the disease activity of SLE [70, 71], and it participates in the pathogenesis of SLE by affecting the IFN pathway in monocytes of SLE [57, 72]. Combined with the results of our study, SIGLEC1$^+$IRF7$^+$ monocytes are expected to be a reliable and easily applicable marker for monitoring disease activity and predicting risk in SLE patients. However, our study also faces another drawback, which is the absence of integration between bulk RNA-seq data and scRNA-seq data. Integrating these data sets will allow us to more robustly validate our findings by linking batching level signals to specific cell types and confirming the cellular origin of key pathways such as the IFN signature. Future studies incorporating these integrations could provide deeper insights into the molecular mechanisms of SLE.

To fortify the credibility of the scRNA-seq analysis, CD14$^+$SIGLEC1$^+$IRF7$^+$ monocytes were concurrently identified in the peripheral blood of both healthy individuals and SLE patients via flow cytometry. The proportion of this particular monocyte subset is elevated in SLE patients and shows a concomitant increase with the progression of disease severity, thereby substantiating the outcomes of the aforementioned scRNA-seq analysis. In addition, both CD14$^+$SIGLEC1$^+$ monocytes and CD14$^+$SIGLEC1$^+$IRF7$^+$ monocytes were able to distinguish SLE from healthy controls, but CD14$^+$SIGLEC1$^+$IRF7$^+$ monocytes were more specific and superior to CD14$^+$SIGLEC1$^+$monocytes in distinguishing the severity of SLE. However, the limited sample size of our study might pose certain limitations.

Besides, our study also lacks an animal model to verify this conclusion. This is due to the extremely difficult validation in mouse models. Firstly, SIGLEC1 is predominantly expressed on CD14$^+$ monocytes in peripheral blood, but only a small proportion of normal monocytes express SIGLEC1, and the diminutive size of mice makes it challenging to detect this specific cell population. Additionally, SIGLEC1 and IRF7 play crucial roles in the IFN-I pathway, and their deletion may result in decreased tolerance to viral infection and ultimately lead to mortality in mice. For example, a study of lymphocytic choriomeningitis virus infection found that the production of IFN-I was reduced after SIGLEC1 deletion, while the mice exhibited severe immunopathology and rapidly died [73]. Therefore, further validation and research from various angles are necessary for the CD14$^+$SIGLEC1$^+$IRF7$^+$ cell population, as well as their involvement in SLE-related mechanisms in the future.

Conclusions

Taken together, our study for the first time identified SIGLEC1$^+$IRF7$^+$ monocytes by scRNA-seq analysis and revealed their importance in SLE. This finding provides new directions for the diagnosis and treatment of SLE and establishes a foundation for future studies on how to utilize monocytes as disease markers and intervention targets. However, further studies are needed to verify its mechanism and function, which will provide more possibilities for personalized treatment of SLE.

Abbreviations

APC	Allophycocyanin
AUC	Area under the curve
AUCell	A tool for single-cell gene set enrichment analysis
B cells	B lymphocytes
CD14	Cluster of differentiation 14
cDCs	Conventional dendritic cells
DAVID	Databases for Annotation, Visualization, and Integrated Discovery
DCs	Dendritic cells
DEGs	Differentially expressed genes
DESeq2	Bioconductor package for differential expression analysis of RNA-seq data
DRESS	Drug-induced hypersensitivity syndrome
FDR	False discovery rate
FITC	Fluorescein isothiocyanate
GEO	Gene Expression Omnibus
GSM	Gene Expression Omnibus Sample
GSE	Gene Expression Omnibus Series
GO	Gene Ontology
GSEA	Gene Set Enrichment Analysis
GSVA	Gene Set Variation Analysis
IFN	Interferon
IFN-I	Type I interferon
IFN-α	Type I interferon alpha
IFN-β	Type I interferon beta
IFN-γ	Type I interferon gamma
IFIT	Interferon-induced protein with tetratricopeptide repeats
IRF7	Interferon regulatory factor 7
ISGs	Interferon-stimulated genes
JAK	Janus Kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
Limma	Linear Models for Microarray Data
LN	Lupus nephritis
Log2FC	Log2 fold change
MHC	Major histocompatibility complex

mAbs	monoclonal antibodies
NES	Normalized enrichment score
NK cells	Natural killer cells
PBMC	Peripheral blood mononuclear cell
PE	Phycoerythrin
pDC	Plasmacytoid dendritic cell
PCA	Principal component analysis
PPI	Protein-protein interaction
RA	Rheumatoid arthritis
RRA	Robust rank aggregation
scRNA-Seq	Single-cell RNA sequencing
SLE	Systemic lupus erythematosus
SLEDAI	SLE Disease Activity Index
STAT1	Signal transducer and activator of transcription 1
T cells	T lymphocytes
Tregs	Regulatory T cells
UMAP	Uniform manifold approximation and projection
UMI	Unique molecular identifier
USP18	Ubiquitin specific peptidase 18

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03560-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

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Author contributions
JM. Z contributed to experimental operations, initial draft, and manuscript editing. WW. Z contributed to data collection and analysis. Y. L contributed to data analysis and interpretation. CQ. D contributed to figure preparation. JX. X contributed to manuscript editing. YC. S contributed to the literature review and drafted and edited the manuscript. Y. H contributed to the literature review, manuscript editing, and experimental design. All authors read and approved the submitted manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
The study has been approved by the First Affiliated Hospital of Xiamen University ethics committee 2024Scientific Research Ethics Approval (218). All participants provided informed consent prior to their inclusion in the study.

Consent for publication
All participants have approved the publication of this work.

Competing interests
The authors declare no competing interests.

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References

1. Pan L, Lu MP, Wang JH, Xu M, Yang SR. Immunological pathogenesis and treatment of systemic lupus erythematosus. World J Pediatrics: WJP Feb. 2020;16(1):19–30.
2. Siegel CH, Sammaritano LR. Systemic lupus erythematosus: A review. Jama May. 2024;7(17):1480–91.
3. Lazar S, Kahlenberg JM. Systemic lupus erythematosus: new diagnostic and therapeutic approaches. Annu Rev Med Jan. 2023;27:74:339–52.
4. Xu H, Zhang X, Wang X, et al. Cellular spermine targets JAK signaling to restrain cytokine-mediated autoimmunity. Immun Aug. 2024;13(8):1796–e18111798.
5. Peyronel F, Rossi GM, Palazzini G, et al. Early-onset lupus nephritis. Clin Kidney J Aug. 2024;17(8):sfae212.
6. Geng W, Zhang S, Cao J, et al. Predictive factors of psychiatric syndrome in patients with systemic lupus erythematosus. Front Immunol. 2024;15:1323209.
7. Psarras A, Wittmann M, Vital EM. Emerging concepts of type I interferons in SLE pathogenesis and therapy. Nat Rev Rheumatol Oct. 2022;18(10):575–90.
8. Zhou Y, Song HM. Type I interferon pathway in pediatric systemic lupus erythematosus. World J Pediatr Jul. 2024;20(7):653–68.
9. Qin Y, Ma J, Vinuesa CG. Monogenic lupus: insights into disease pathogenesis and therapeutic opportunities. Curr Opin Rheumatol May. 2024;1(3):191–200.
10. Postal M, Vivaldo JF, Fernandez-Ruiz R, Paredes JL, Appenzeller S, Niewold TB. Type I interferon in the pathogenesis of systemic lupus erythematosus. Curr Opin Immunol Dec. 2020;67:87–94.
11. Arish M, Sun J. Monocyte and macrophage function in respiratory viral infections. Anim Dis 2023/09/14. 2023;3(1):30.
12. Shen M, Duan C, Xie C, et al. Identification of key interferon-stimulated genes for indicating the condition of patients with systemic lupus erythematosus. Front Immunol. 2022;13:962393.
13. Perez RK, Gordon MG, Subramaniam M, et al. Single-cell RNA-seq reveals cell type-specific molecular and genetic associations to lupus. Sci (New York N Y) Apr. 2022;8(6589):eabf1970.
14. Carter LM, Wigston Z, Laws P, Vital EM. Rapid efficacy of anifrolumab across multiple subtypes of recalcitrant cutaneous lupus erythematosus parallels changes in discrete subsets of blood transcriptomic and cellular biomarkers. Br J Dermatol Jul. 2023;17(2):210–8.
15. Lv F, He Y, Xu H, et al. CD36 aggravates podocyte injury by activating NLRP3 inflammasome and inhibiting autophagy in lupus nephritis. Cell Death Disease Aug. 2022;23(8):729.
16. Tsai CY, Li KJ, Shen CY et al. Decipher the immunopathological mechanisms and set up potential therapeutic strategies for patients with lupus nephritis. Int J Mol Sci Jun 13 2023;24(12).
17. Kong R, Sun L, Li H, Wang D. The role of NLRP3 inflammasome in the pathogenesis of rheumatic disease. Autoimmun Feb. 2022;55(1):1–7.

18. Zhang Y, Liao Y, Hang Q, Sun D, Liu Y. GBP2 acts as a member of the interferon signalling pathway in lupus nephritis. BMC Immunol Sep. 2022;17(1):44.
19. Liu N, Gao Y, Liu Y, Liu D. GBP5 Inhibition ameliorates the progression of lupus nephritis by suppressing NLRP3 inflammasome activation. Immunological Investigations Sep. 2022;29:1–15.
20. Liu J, Zhang X, Cao X. Dendritic cells in systemic lupus erythematosus: from pathogenesis to therapeutic applications. J Autoimmun Oct. 2022;132:102856.
21. Nehar-Belaid D, Hong S, Marches R, et al. Mapping systemic lupus erythematosus heterogeneity at the single-cell level. Nat Immunol Sep. 2020;21(9):1094–106.
22. Wang G, Tao H, Zhou L, et al. scRNA-seq reveals involvement of monocytes in immune response in SLE patients. Genomics Jan. 2025;14(2):110994.
23. Sarin KY, Zheng H, Chaichian Y et al. Impaired innate and adaptive immune responses to BNT162b2 SARS-CoV-2 vaccination in systemic lupus erythematosus. JCI Insight Mar 8 2024;9(5).
24. Honardoost MA, Adinatha A, Schmidt F, et al. Systematic immune cell dysregulation and molecular subtypes revealed by single-cell RNA-seq of subjects with type 1 diabetes. Genome Med Mar. 2024;27(1):45.
25. Kolde R, Laur S, Adler P, Vilo J. Robust rank aggregation for gene list integration and meta-analysis. Bioinf (Oxford England) Feb. 2012;15(4):573–80.
26. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol Jun. 2018;36(5):411–20.
27. Aran D, Looney AP, Liu L, et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nat Immunol Feb. 2019;20(2):163–72.
28. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
29. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res Apr. 2015;20(7):e47.
30. Van de Sande B, Flerin C, Davie K, et al. A scalable SCENIC workflow for single-cell gene regulatory network analysis. Nat Protoc Jul. 2020;15(7):2247–76.
31. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinf Jan. 2013;16:14:7.
32. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci United States Am Oct. 2005;25(43):15545–50.
33. Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols.* 2009;4(1):44–57.
34. Spidlen J, Breuer K, Rosenberg C, Kotecha N, Brinkman RR. FlowRepository: a resource of annotated flow cytometry datasets associated with peer-reviewed publications. Cytometry Part A: J Int Soc Anal Cytol Sep. 2012;81(9):727–31.
35. Mokbel A, Fouad NA, Alkemary A, Abdo M. Disease activity at the onset of diagnosis as a predictor of disease outcomes in a cohort of patients with systemic lupus erythematosus: A post hoc retrospective analysis of the COMOSLE-EGYPT study. Clin Rheumatol Jan. 2025;44(1):229–35.
36. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol Feb. 2002;29(2):288–91.
37. Ambler WG, Kaplan MJ. Vascular damage in systemic lupus erythematosus. Nat Rev Nephrol Apr. 2024;20(4):251–65.
38. Waterman HR, Dufort MJ, Posso SE, et al. Lupus IgA1 autoantibodies synergize with IgG to enhance plasmacytoid dendritic cell responses to RNA-containing immune complexes. Sci Transl Med Jul. 2024;3(754):eadl3848.
39. Infante B, Mercuri S, Dello Strologo A et al. Unraveling the link between Interferon-α and systemic lupus erythematosus: from the molecular mechanisms to target therapies. Int J Mol Sci Dec 15 2022;23(24).
40. Xu Y, Li P, Li K, et al. Pathological mechanisms and crosstalk among different forms of cell death in systemic lupus erythematosus. J Autoimmun Oct. 2022;132:102890.
41. Sim TM, Ong SJ, Mak A, Tay SH. Type I interferons in systemic lupus erythematosus: A journey from bench to bedside. Int J Mol Sci Feb 24 2022;23(5).
42. Sandling JK, Pucholt P, Hultin Rosenberg L, et al. Molecular pathways in patients with systemic lupus erythematosus revealed by gene-centred DNA sequencing. Annals Rheumatic Dis Jan. 2021;80(1):109–17.
43. Zhang Y, Wang J, Fang Y, et al. IFN-α affects Th17/Treg cell balance through c-Maf and associated with the progression of EBV- SLE. Mol Immunol Jul. 2024;171:22–35.
44. Kiefer K, Oropallo MA, Cancro MP, Marshak-Rothstein A. Role of type I interferons in the activation of autoreactive B cells. Immunol Cell Biology May. 2012;90(5):498–504.
45. Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. Nat Rev Immunol Aug. 2015;15(8):471–85.
46. Alculumbre S, Raieli S, Hoffmann C, Chelbi R, Danlos FX, Soumelis V. Plasmacytoid pre-dendritic cells (pDC): from molecular pathways to function and disease association. Semin Cell Dev Biol Feb. 2019;86:24–35.
47. Hamilton JA, Hsu HC, Mountz JD. Autoreactive B cells in SLE, villains or innocent bystanders? Immunol Rev Nov. 2019;292(1):120–38.
48. Wang L, Yang F, Ye J, Zhang L, Jiang X. Insight into the role of IRF7 in skin and connective tissue diseases. Exp Dermatol May. 2024;33(5):e15083.
49. Caielli S, Balasubramanian P, Rodriguez-Alcazar J et al. An unconventional mechanism of IL-1β secretion that requires Type I IFN in lupus monocytes. *bioRxiv.* Aug 3. 2023.
50. Flint SM, Jovanovic V, Teo BW, et al. Leucocyte subset-specific type 1 interferon signatures in SLE and other immune-mediated diseases. RMD Open. 2016;2(1):e000183.
51. Mai L, Asaduzzaman A, Noamani B, et al. The baseline interferon signature predicts disease severity over the subsequent 5 years in systemic lupus erythematosus. Arthritis Res Ther Jan. 2021;16(1):29.
52. Drougkas K, Skarlis C, Mavragani C. Type I interferons in systemic autoimmune rheumatic diseases: pathogenesis, clinical features and treatment options. Mediterr J Rheumatol Jun. 2024;35(Suppl 2):365–80.
53. Strauss R, Rose T, Flint SM, et al. Type I interferon as a biomarker in autoimmunity and viral infection: a leukocyte subset-specific analysis unveils hidden diagnostic options. J Mol Med (Berl) Jul. 2017;95(7):753–65.
54. Villani AC, Satija R, Reynolds G et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Sci Apr 21 2017;356(6335).
55. Dutertre CA, Becht E, Irac SE, et al. Single-Cell analysis of human mononuclear phagocytes reveals Subset-Defining markers and identifies Circulating inflammatory dendritic cells. Immun Sep. 2019;17(3):573–e589578.
56. Renaudineau Y, Charras A, Natoli V, et al. Type I interferon associated epistasis May contribute to early disease-onset and high disease activity in juvenile-onset lupus. Clin Immunol (Orlando Fla) May. 2024;262:110194.
57. Ma W, Huang G, Wang Z, Wang L, Gao Q. IRF7: role and regulation in immunity and autoimmunity. Front Immunol. 2023;14:1236923.
58. Minaga K, Watanabe T, Arai Y, et al. Activation of interferon regulatory factor 7 in plasmacytoid dendritic cells promotes experimental autoimmune pancreatitis. J Gastroenterol May. 2020;55(5):565–76.
59. Stuckrad SLV, Klotsche J, Biesen R, et al. SIGLEC1 (CD169) is a sensitive biomarker for the deterioration of the clinical course in childhood systemic lupus erythematosus. Lupus Dec. 2020;29(14):1914–25.
60. Eakin AJ, Ahmed T, McGeough CM et al. CD169 + Monocyte and regulatory T cell subsets are associated with disease activity in rheumatoid arthritis. J Pers Med Nov 9 2022;12(11).
61. Xu L, Huang C, Zheng X, et al. Elevated CD169 expressing monocyte/macrophage promotes systemic inflammation and disease progression in cirrhosis. Clin Exp Med Feb. 2024;28(1):45.
62. Affandi AJ, Olesek K, Grabowska J, et al. CD169 defines activated CD14(+) monocytes with enhanced CD8(+) T cell activation capacity. Front Immunol. 2021;12:697840.
63. Jian S, Li H. Peripheral mononuclear cells and systemic lupus erythematosus association: integrated study of single-cell sequencing and Mendelian randomization analysis. Lupus Oct. 2024;15:9612033241292705.
64. Guo C, Liu Q, Zong D, et al. Single-cell transcriptome profiling and chromatin accessibility reveal an exhausted regulatory CD4 + T cell subset in systemic lupus erythematosus. Cell Rep Nov. 2022;8(6):111606.
65. Carvalheiro T, Rodrigues A, Lopes A, et al. Tolerogenic versus inflammatory activity of peripheral blood monocytes and dendritic cells subpopulations in systemic lupus erythematosus. Clin Dev Immunol. 2012;2012:934161.
66. Rose T, Grützkau A, Klotsche J et al. Are interferon-related biomarkers advantageous for monitoring disease activity in systemic lupus erythematosus? A longitudinal benchmark study. *Rheumatology (Oxford).* Sep 1. 2017;56(9):1618–1626.
67. Szabó E, Faragó A, Bodor G, et al. Identification of immune subsets with distinct lectin binding signatures using multi-parameter flow cytometry: correlations with disease activity in systemic lupus erythematosus. Front Immunol. 2024;15:1380481.

68. Rose T, Grutzkau A, Hirseland H, et al. IFNalpha and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. Ann Rheum Dis Oct. 2013;72(10):1639–45.
69. Alexander T, Sarfert R, Klotsche J, et al. The proteasome inhibitior bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. Ann Rheum Dis Jul. 2015;74(7):1474–8.
70. Fu Q, Zhao J, Qian X, et al. Association of a functional IRF7 variant with systemic lupus erythematosus. Arthritis Rheum Mar. 2011;63(3):749–54.
71. Lin LH, Ling P, Liu MF. The potential role of interferon-regulatory factor 7 among Taiwanese patients with systemic lupus erythematosus. J Rheumatol Sep. 2011;38(9):1914–9.
72. Xu WD, Zhang YJ, Xu K, et al. IRF7, a functional factor associates with systemic lupus erythematosus. Cytokine Jun. 2012;58(3):317–20.
73. Shaabani N, Duhan V, Khairnar V, et al. CD169(+) macrophages regulate PD-L1 expression via type I interferon and thereby prevent severe immunopathology after LCMV infection. Cell Death Dis Nov. 2016;3(11):e2446.

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