Belimumab modulates type I interferon signalling in the treatment of systemic lupus erythematosus

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Abstract

Objective

To examine the effects of belimumab on the immune atlas in patients with systemic lupus erythematosus (SLE).

Methods

We present a single-cell RNA-seq profile of peripheral blood mononuclear cells from six patients with active SLE before and after drug treatment initiation. Three of these patients received belimumab combined with conventional therapy, while three received conventional therapy alone, and served as the control group.

Results

We found that belimumab significantly decreased the number of CD16+ monocytes after 8 weeks of treatment, whereas the opposite was observed in the control group. Compared to conventional therapy, belimumab elicited a significant reduction in IFN-stimulated gene (ISG) activity in monocytes and low-density granulocytes (LDGs). Notably, the expansion of unique subpopulations enriched among ISGs was inhibited in patients treated with belimumab. Moreover, the transcription and expression of BAFF-R and B-cell maturation antigen, two BAFF receptors, was increased in plasmacytoid dendritic cells (pDCs), B cells and plasma cells. However, the expression of BAFF-R was inhibited in monocytes and T cells in patients with SLE.

Conclusion

These results revealed a novel mechanism of belimumab action, advancing our understanding of the immune atlas in SLE patients before and after belimumab-targeting treatment.

Key words

systemic lupus erythematosus, B-cell activating factor, type I interferon, belimumab, B-cell activating factor receptors

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Introduction

Systemic lupus erythematosus (SLE) is an idiopathic autoimmune disease characterised by the breakdown of tolerance against nucleic acids, resulting in the production of autoantibodies, organ inflammation, and tissue damage (1). The clinical manifestations of SLE are complex and unpredictable, with periods of flare-ups alternating with phases of remission, which lead to cumulative damage (2). After years without targeted therapeutic modalities, biological agents have emerged as treatment options for SLE over recent decades.

Belimumab, the first biological agent approved for the treatment of SLE, has demonstrated efficacy against this condition in both clinical trials and real-world settings (3, 4). Belimumab specifically binds to the soluble form of B-cell activating factor (BAFF), also known as B lymphocyte stimulator, and inhibits its activity. BAFF promotes B-cell survival and maturation via three receptors expressed on the surface of B cells-BAFF receptor (BAFF-R), B-cell maturation antigen (BCMA), and transmembrane activator and cyclophilin ligand interactor (TACI) (5). Clinical trials and *post-hoc* analysis of data obtained from SLE patients treated with belimumab have suggested that mechanisms other than B-cell depletion are affected in response to belimumab therapy (6, 7). In addition, other cell subsets, such as follicular helper T cells (Tfh), cytotoxic T lymphocytes, and monocytes, also express different BAFF receptors, their presence and function on these immune cells, contribute to the broader immune landscape, influencing both adaptive and innate immune responses (8-10), indicating that belimumab may regulate the function of other immune cells in addition to B cells.

Aside from BAFF, type I interferons (IFN-Is), particularly IFN-α, are also strongly associated with the development of SLE. Several studies have indicated that elevated expression levels of IFN-stimulated genes (ISGs), referred to as the IFN signature, are correlated with lupus disease activity (11-13). Strikingly, BAFF expression is directly linked to and upregulated by ISGs (14). The activity of IFNα in the serum of patients with SLE was observed to be positively correlated with circulating BAFF levels (15). Furthermore, improved responses to belimumab have been reported in SLE patients with high mRNA levels of IFN-I or BAFF at baseline (16). Combined, these studies showed that the functions of BAFF and IFNI are closely connected. However, data concerning the potential effect of the targeted blockade of BAFF on the IFN-I signature during belimumab treatment in SLE remains limited.

In the current study, we performed a cross-sectional analysis of SLE patients, monitoring relevant cellular responses before and after belimumab administration. Single-cell RNA sequencing (scRNA-seq) was undertaken on peripheral blood mononuclear cells (PBMCs) from patients with SLE receiving belimumab combined with standard treatment or standard treatment alone. We identified and compared cell-type- and subpopulation-specific changes in the expression of immune cell marker genes, as well as alterations in the IFN signature after *versus* before belimumab therapy. This study uncovered the immune cell and molecular profiles associated with IFN signalling after belimumab treatment and shed light on the mechanisms of action and potential applications of BAFF inhibitors.

Materials and methods

Clinical samples

Three SLE patients commencing belimumab treatment and three SLE controls, matched as closely as possible for disease activity, gender, and ethnicity, were recruited from Nanfang Hospital, an affiliated academic hospital of Southern Medical University. All the patients received mycophenolate mofetil (MMF) therapy as the standard treatment (ST). As an add-on to ST, three patients were given belimumab (10 mg/kg) intravenously once every 2 weeks for the first 6 weeks, and then once a week 8, for a total of four doses. This study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Nanfang Hospital (no. NFEC-2020-204). Informed consent was obtained

Table I. Demographic and clinical information of the study cohort.

Demographics	ST (n=3)	ST + BEL (n=3)
Age (years)	32.05 ± 21.16	35.16 ± 15.79
Females, n (%)	3 (100%)	3 (100%)
Asian ethnicity, n (%)	3 (100%)	3 (100%)
Lupus history		
Disease duration (months)	26.31 ± 12.63	20.24 ± 15.97
SLEDAI score	9.2 ± 4.53	10.4 ± 3.76
Cutaneous manifestations	1 (33%)	2 (67%)
Articular involvement (arthritis/Jaccoud's arthropathy)	3 (100%)	2 (67%)
Renal involvement	1 (33%)	1 (33%)
Anti-dsDNA autoantibodies at baseline (IU/mL)	290.19 ± 212.07	348.99 ± 124.16
C3 at baseline (g/L)	0.56 ± 0.24	0.42 ± 0.19
C4 at baseline (g/L)	0.09 ± 0.04	0.06 ± 0.02
Medication		
Mycophenolate mofetil	3 (100%)	3 (100%)
Prednisone (mg/day)	13.6 ± 8.9	11.2 ± 6.4

from all participants. The exclusion criteria included acute infection, pregnancy, cancer or other malignant disease, or other connective tissue disease.

Single-cell transcriptomic analysis

PBMCs were isolated from SLE patients by Ficoll-Paque gradient centrifugation (Solarbio, Beijing, China) before and 8 weeks after receiving belimumab + ST or ST alone. After washing with PBS supplemented with 0.1% BSA, cell numbers and viability were determined using Trypan blue staining. Single-cell capture and library preparation were performed using the BD Rhapsody Single-Cell Analysis System (BD, USA) according to the manufacturer's instructions. The resulting libraries were sequenced over multiple runs of the NovaSeq platform (Illumina, San Diego, CA, USA).

Processing of raw single-cell sequencing data

The sequencing data were processed and examined through the BD Rhapsody Whole Transcriptome Analysis pipeline. The Seurat R package (v. 3.2.2) was applied for unsupervised clustering and visualisation (17). Genes expressed in fewer than two cells were filtered out. Cells expressing more than 200 genes and with a mitochondrial gene content below 10% were retained for further analysis. Dimensionality reduction of the filtered data was performed *via* principal component analysis (PCA) of the 1,500 genes showing the highest variation in expression. Fifty significant principal components were used as the input for subsequent uniform manifold approximation and projection processing. Cells were clustered using the FindClusters function in the Seurat package. Subsequently, the FindAllMarkers function was used to identify differentially expressed genes (DEGs) in each cell type or subtype. Major cell types were annotated based on the top DEGs in each cluster and each gene was manually checked on the CellMarker database.

IFN signature scoring

ISGs from three previously described IFN-related modules were used to calculate the IFN signature in each cluster (18). The unique molecular identifier (UMI) count for each gene was normalised by the total number of UMIs per cell and converted to transcripts-per-10,000 (henceforth 'TP10K') values. IFN signature scores were calculated as the mean log2 (TP10K+1) across all genes in the signature.

Quantitative PCR

Monocytes, B cells, T cells, plasmacytoid dendritic cells (pDCs), and plasma cells were isolated from PBMCs by direct magnetic labelling with microbeads conjugated with anti-CD14, anti-CD19, anti-CD3, anti-CD304, and anti-CD138 antibodies (Miltenyi Biotec, Germany), respectively. Cell purity routinely exceeded 90% while viability ranged from 92% to 95%, as determined by flow cytometry. RNA was isolated from these cell subpopulations using the RNeasy Micro Kit (Qiagen, Venlo, the Netherlands). cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). For quantitative PCR, TaqMan assays (Applied Biosystems) were used in combination with the TaqMan PCR Core Reagent Kit (Applied Biosystems). The primers used for the amplification of human *BCMA*, *TACI*, and *BAFF-R* have been previously described (19).

Statistics

Statistical analysis was performed using R/4.1.2. The Student's *t*-test was used to estimate the significance of differences between the two groups. Differences between before and after treatment were evaluated using paired-sample t-tests or paired-sample Wilcoxon signed rank tests. A *p*-value lower than 0.05 was considered statistically significant.

Results

Belimumab affected the SLEDAI-2K scores, C3 and C4 levels in SLE patients

Demographic and clinical information for the SLE patients are summarised in Table I. Three patients received only MMF (ST group) and three received MMF combined with belimumab (ST-B group) (Fig. 1A). At baseline, no statistically significant differences were found between the two groups regarding general conditions and disease indicators, including SLEDAI-2K scores, C3 and C4 levels, and laboratory tests ($p>0.05$). After 8 weeks of treatment, the SLEDAI-2K scores were significantly decreased in both groups of patients (Fig. 1B, F), accompanied by significant decreases in anti-dsDNA auto-antibody titres (Fig. 1E, I) and marked improvements in serum levels of complement C3 (Fig. 1C, G) and C4 (Fig. 1D, H). Furthermore, the parameters of SLEDAI-2K scores, C3 and C4 levels differed significantly (Fig-1J-M) between the ST and ST-B groups after 8 weeks of treatment in SLE patients. Overall, these results suggested that the short-term effects of belimumab on the above-mentioned parameters were comparable to those of ST.

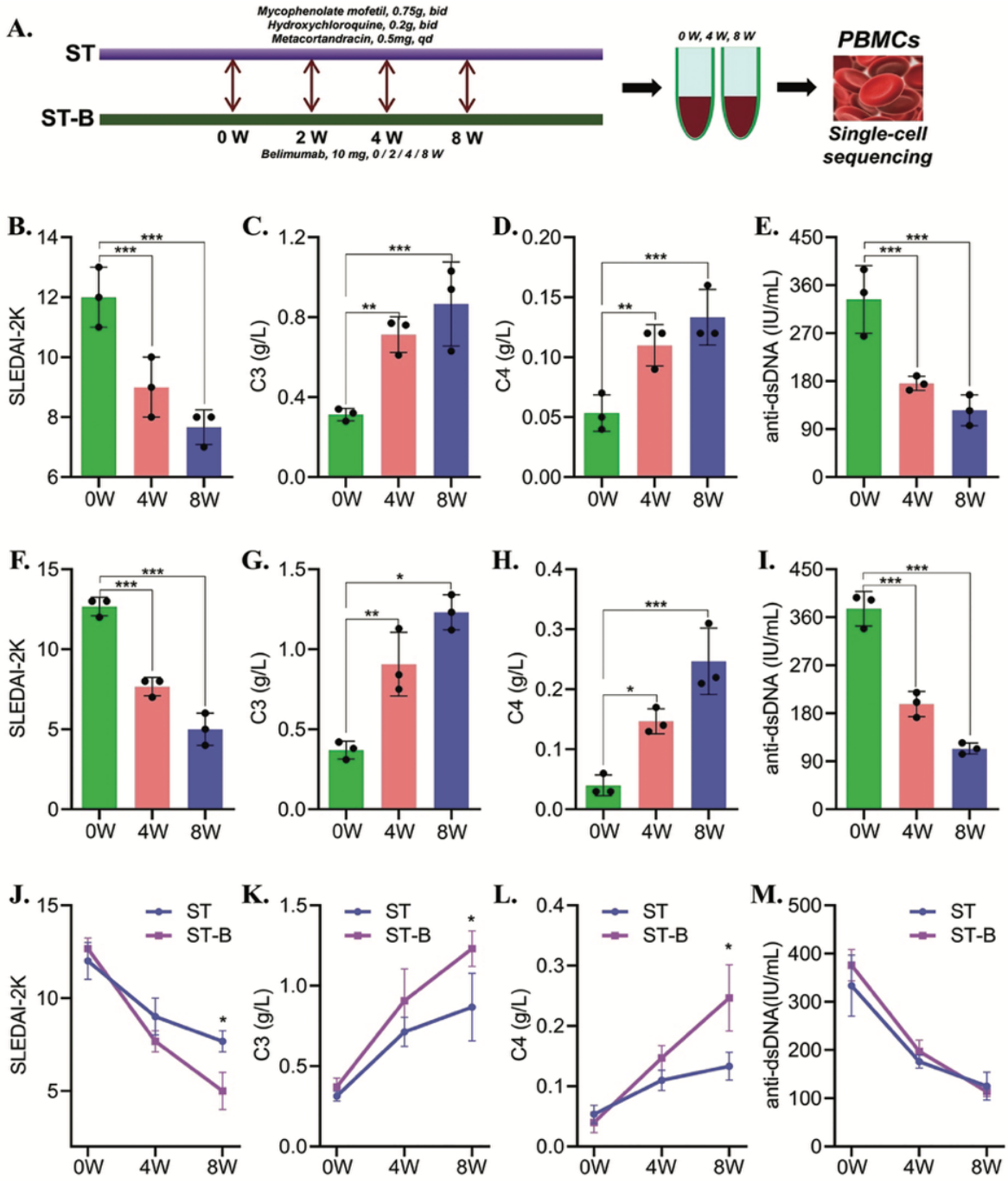

Fig. 1. Belimumab affected the SLEDAI-2K scores, C3 and C4 levels in SLE patients.
(**A**) Schematic representation of the experimental workflow. Comparisons of the clinical and laboratory features of patients with systemic lupus erythematosus (SLE) who received standard treatment (ST group) or standard treatment in combination with belimumab (ST-B group) after 8 weeks of treatment. Compared to baseline, belimumab significantly improved the disease activity index 2000 (SLEDAI-2K) score (**B** and **F**) and the C3 (**C** and **G**) and C4 (**D** and **H**) levels, while the concentrations of anti-double-stranded DNA autoantibodies (anti-dsDNA) (**E** and **I**) showed a significant downward trend. Inside, Figure 1 **B-E** is the results of ST group; Figure 1 **F-I** is the results of the ST-B group. Comparison of the SLEDAI-2K score (**J**) and the C3 (**K**), C4 (**L**), and anti-dsDNA (**M**) levels in the two groups of SLE patients.
*p<0.05, **p<0.01, ***p<0.001.

Belimumab affected the complexity of PBMCs in SLE patients

We analysed the transcriptomes of 65,543 PBMCs obtained from the six SLE patients (three from the ST group and three from the ST-B group) before and after the respective treatments using microwell-based scRNA-seq. Following the application of quality-control filters, a total of 63,535 cells were obtained for further analysis. Among these, 14 molecularly distinct populations were identified by unsupervised clustering after correction of batch effects and principal component analyses (PCA) (Fig. 2A). The clusters were assigned to known cell lineages based on the differential expression of marker genes

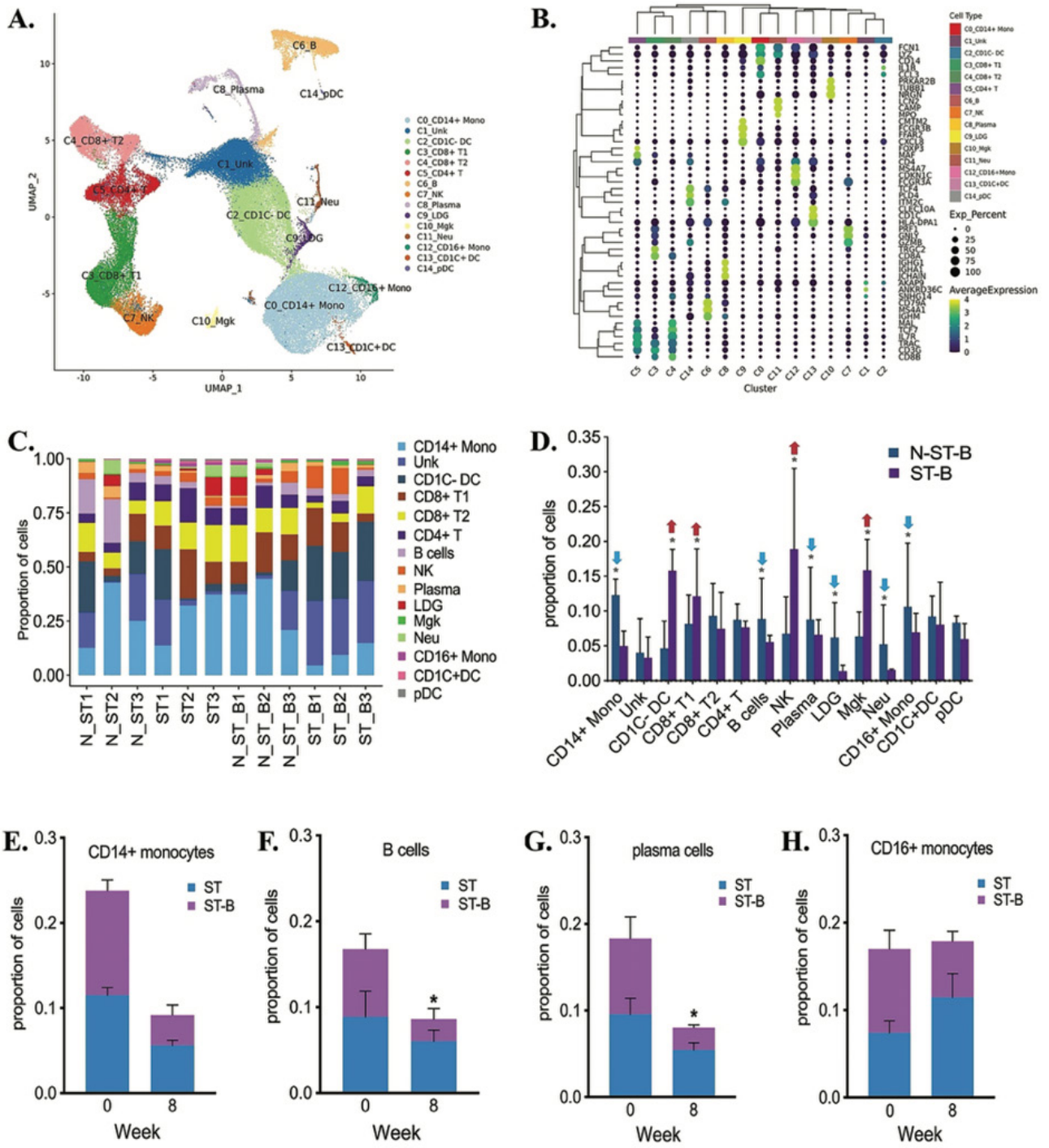

Fig. 2. Belimumab affected the complexity of PBMCs in SLE patients.
(**A**) The single-cell transcriptomic profiles of PBMCs from patients with SLE before and after mycophenolate mofetil (MMF) or MMF + belimumab treatment. The 15 identified clusters are shown as a uniform manifold approximation and projection (UMAP) plot. (**B**) A dot plot of marker genes across each identified cluster. (**C**) A bar plot representing the proportions of each cell type in each sample. (**D**) Comparison of the proportions of each cluster after belimumab treatment. For each cell type, red arrows indicate significant upregulation and blue arrows denote significant downregulation. (**E**-**H**) The percentage of CD14+ monocytes (**E**), B cells (**F**), plasma cells (**G**), and CD16+ monocytes (**H**) before and after treatment in the two groups.
*p<0.05. N-ST: before standard treatment with MMF; ST: standard treatment with MMF; N-ST-B: before therapy with belimumab and MMF; ST-B: belimumab combined with MMF.

relative to all other cells (Fig. 2B). One cluster, C1-Unk, was not clearly identifiable. The remaining 13 cell lineages were B cells, CD4$^+$ T cells, natural killer (NK) cells, plasma cells, low-density granulocytes (LDGs), megakaryocytes (Mgks), neutrophils, pDCs, two clusters of conventional dendritic cells (cDCs), CD8$^+$ T cells, and monocytes (CD14$^+$ and CD16$^+$).

The proportions of these clusters varied greatly among individuals in both groups (Fig. 2C). First, we analysed the changes in cellular composition by comparing the frequencies of dif-

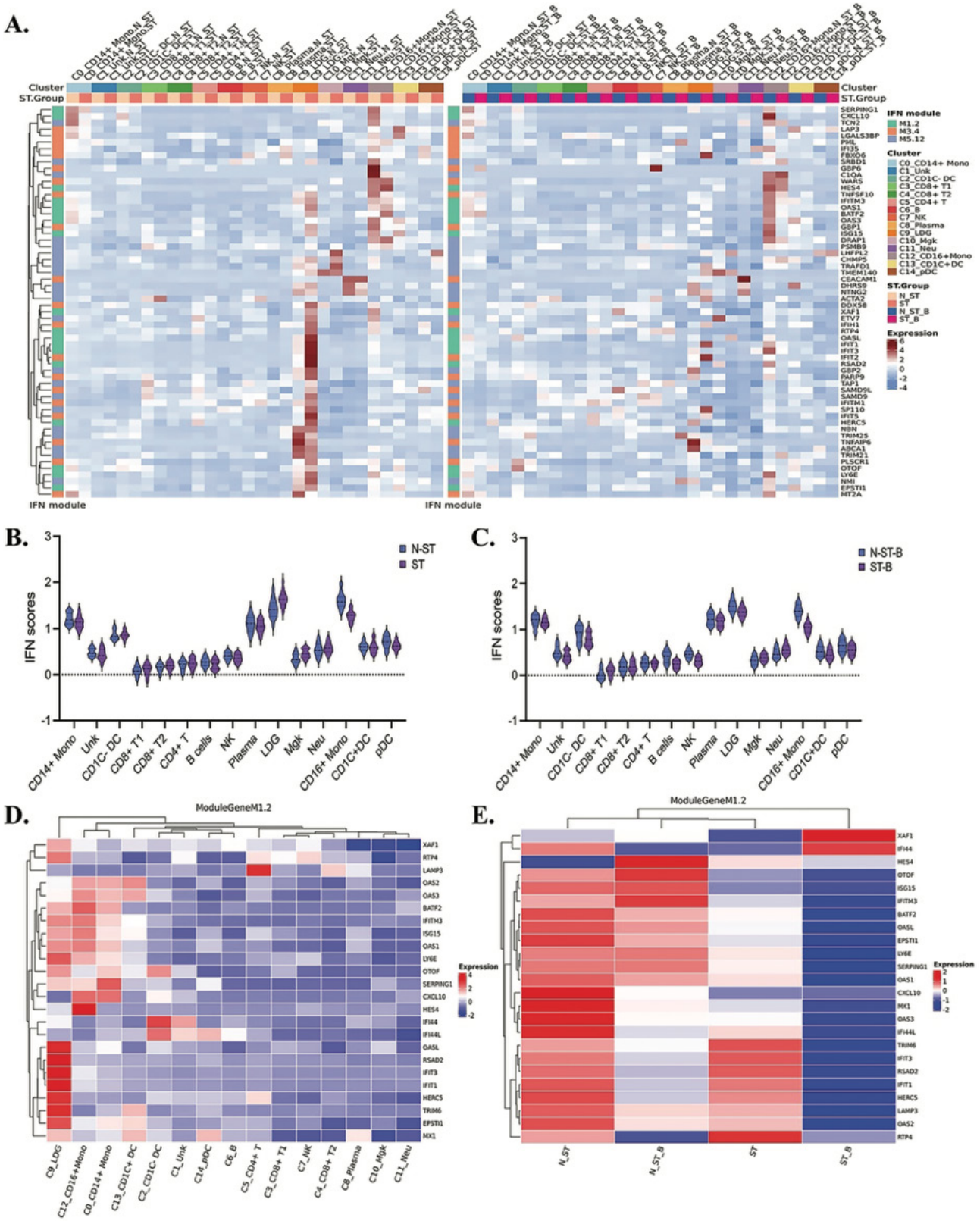

Fig. 3. Belimumab affected the IFN-response signature in clusters of peripheral blood mononuclear cells (PBMCs) from patients with systemic lupus erythematosus (SLE).
(**A**) The effect of belimumab on the IFN-stimulated gene (ISG) scores of the cell clusters from SLE patients. Heatmaps representing the mean expression of ISGs (n=60 unique genes) from IFN-related modules across the clusters (n=15) before and after treatment in the two groups. Left: comparison of ISG expression after 8 weeks of standard treatment with mycophenolate mofetil (MMF). Right: changes in ISG expression in SLE patients treated with belimumab + MMF. (**B**-**C**) Violin plots of the IFN signature scores for cell components of each cluster between the MMF-only treatment (**B**) and belimumab + MMF treatment (**C**) groups. (**D**) Heatmap of the ISGs from module M1.2 across the 15 clusters. (**E**) Heatmap of ISGs from module M1.2 between different groups.

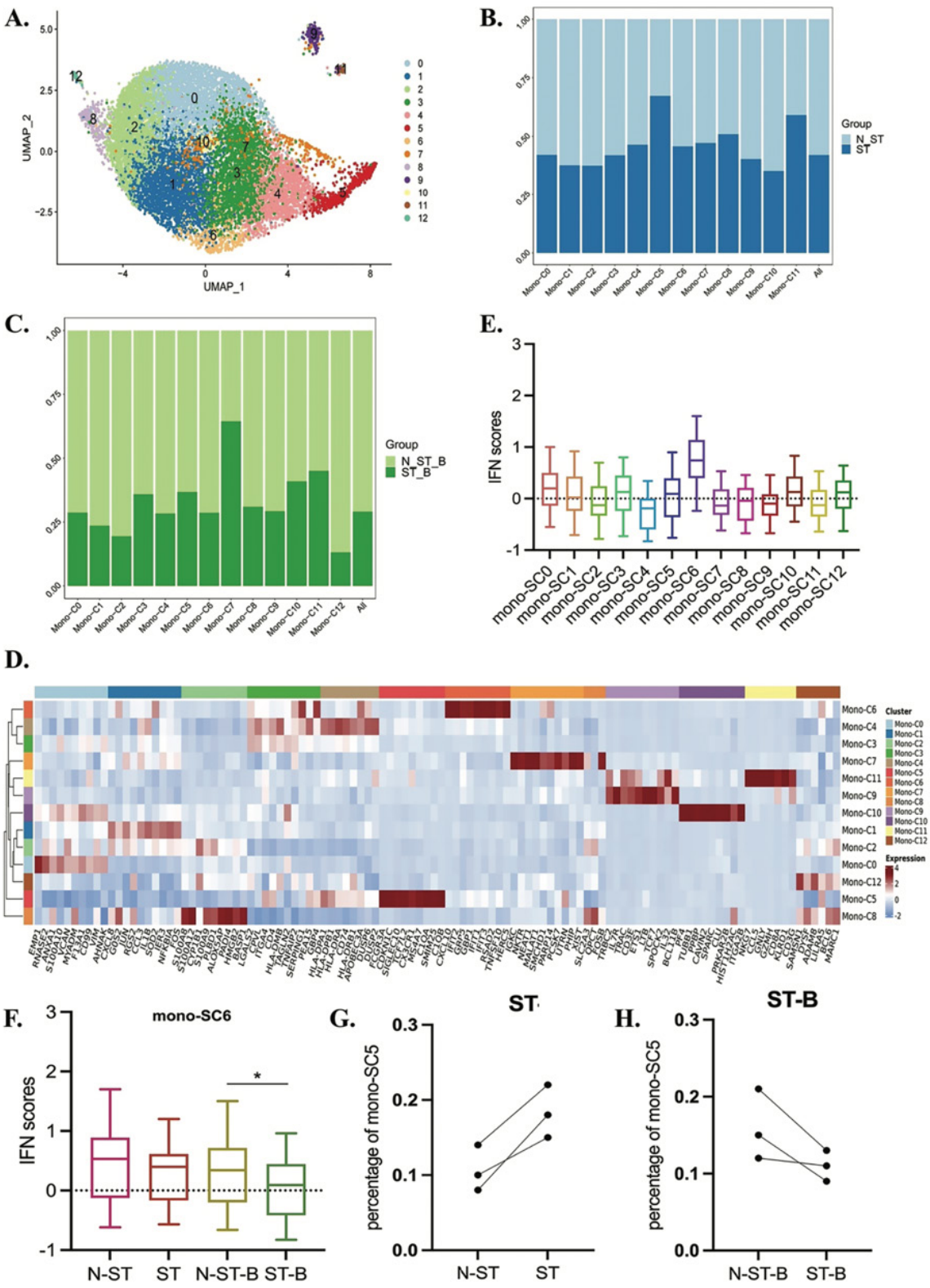

Fig. 4. Belimumab affected the IFN-I pathway in monocyte subclusters.
(**A**) Alterations in the proportions of monocyte clusters after standard treatment or standard treatment + belimumab. Shown is a uniform manifold approximation and projection (UMAP) plot representing monocyte subclusters (mono-SCs, n=13) from patients with systemic lupus erythematosus (SLE). (**B**) A bar plot highlighting cell abundances across mono-SCs for pre-standard treatment (N-ST group) *versus* post-standard treatment (ST group). (**C**) Cell abundances across mono-SCs for pre-standard treatment + belimumab (N-ST-B group) and post-standard treatment + belimumab (ST-B group). (**D**) A heatmap displaying the expression of genes defining each of the mono-SCs. (**E**) IFN scores for each mono-SC (**F**). IFN signature scores for mono-SC6 in the different groups after 8 weeks of treatment. (**G**, **H**) Changes in the percentage of mono-SC5 in individual samples from the ST group (**G**) and ST-B group (**H**) after 8 weeks of therapy (*p<0.05).

ferent cell types before and after belimumab and MMF co-treatment in the ST-B group. The results showed that subsets under-represented after treatment included $CD14^+$ monocytes, B cells, plasma cells, LDGs, neutrophils, and $CD16^+$ monocytes ($p<0.05$). Subsets that were over-represented in SLE patients included CD1c-DCs, $CD8^+$ T1 cells, NK cells, and Mgks ($p<0.05$) (Fig. 2D). The proportions of the remaining subsets were similar before and after therapy in the ST-B group. Second, we assessed the effects of the two treatment regimens on the proportion of PBMCs from SLE patients. The proportions of $CD14^+$ monocytes (Fig. 2E), B cells (Fig. 2F), and plasma cells (Fig. 2G) were significantly reduced relative to baseline in patients from the ST group and the reductions in the proportions of these cell subsets were less pronounced in these patients than in those from the ST-B group. However, the proportions of $CD16^+$ monocytes without changed between the ST and ST-B group. (Fig. 2H). Moreover, the proportions of $CD16^+$ monocytes, which were expanded in the ST group, were reduced in the ST-B group after 8 weeks of treatment.

Belimumab affected the IFN-response signature in SLE PBMCs clusters

Changes in circulating leukocyte ratios as well as the association of more potent therapies with significant decrease in plasmablast and IFN response. Given the importance of the IFN-I signature in the pathogenesis of SLE, we analysed the single-cell transcriptomic profiles of the PBMCs based on a previously identified framework comprising three IFN modules (M1.2, M3.4, and M5.12) associated with disease activity (18, 20). After conventional treatment with MMF (ST group), the expression of ISGs was decreased in the $CD16^+$ monocyte subset ($C12^-CD16^+$Mono) but remained largely unchanged in the other cell clusters (Fig. 3A). However, the ISGs displaying high expression in $CD16^+$ monocytes were effectively downregulated after 8 weeks of belimumab therapy (ST-B group). Notably, compared to baseline, the expression of ISGs in the LDG cluster (C9_LDG) increased in patients of the ST group after 8 weeks of conventional therapy. In contrast, no differences in the expression of ISGs in LDGs relative to baseline were observed in the ST-B group. These results were in line with the IFN scores, obtained based on the average expression of ISGs from the three modules in each cell (Fig. 3B-C).

As the three IFN modules displayed distinct activation thresholds (M1.2 <M3.4 <M5.12), we also assessed the changes in ISG expression in individual modules before and after MMF or MMF + belimumab treatment. The LDG and monocyte clusters contributed the most to the upregulation of M1.2 genes (Fig. 3D), and this effect was more pronounced in PBMCs from patients in the ST-B group than in those from the ST group (Fig. 3E). Genes from modules M3.4 and M5.12 displayed more complex regulation in both groups, with some being upregulated and some downregulated after 8 weeks of treatment. The ISGs of the three modules were mainly expressed in $CD14^+$ monocytes, $CD16^+$ monocytes and LDGs.

Belimumab affected the IFN-I pathway in monocyte subclusters

Monocyte dysfunction is known to play a crucial role in SLE immunopathogenesis. In this study, scRNA-seq analysis yielded two major monocyte subsets ($CD14^+$ and $CD16^+$) comprising 13,588 cells. A second round of clustering produced 13 monocyte subclusters (mono-SCs), two of which (mono-SC5 and mono-SC11) were expanded in patients of the ST group. Additionally, except for mono-SC7, the proportions of most mono-SCs were decreased in samples from the ST-B group (Fig. 4A-C). Of note, differential gene expression analysis revealed that the IFN signature was activated in mono-SC6, consistent with the IFN scores for the monocyte subclusters (Fig. 4D). Furthermore, ISG activity in mono-SC6 was not altered after ST alone but was efficiently suppressed after 8 weeks of belimumab + MMF treatment (Fig. 4E-F).

It has been reported that $CD16^+$ monocytes are enriched in PBMCs of patients with SLE (21). Here, we noted that CD16a (FCGR3A) was mainly expressed in mono-SC5 (Fig. 4G-H) after conventional therapy (ST group). In particular, we detected an increase of mono-SC5 in all three patients of the ST group. After belimumab treatment (ST-B group, the percentage of mono-SC5 was decreased in two patients but remained unaltered in the third (Fig. 4G).

Belimumab affected the IFN-I pathway in LDG subclusters

LDGs comprise a proinflammatory neutrophil subset associated with SLE pathogenesis. Given that our findings suggested that MMF in combination with belimumab affected IFN-I signalling in LDGs, we further explored LDG heterogeneity in this study. LDG subclustering (n=630) analysis identified three LDG-SCs (LDG-SC0, 1, and 2) (Fig. 5A), while differential gene expression analysis revealed that IFN-related genes (Fig. 5B) such as *IFIT2* (Fig. 5C), *GBP4* (Fig. 5D), *JUN* (Fig. 5E), and *FCGR3B* (Fig. 5F) displayed varying degrees of activity in all three LDG-SCs. However, the percentages of LDG-SC1 and SC2 cells were markedly reduced after treatment with belimumab (ST-B group), whereas the opposite trend was observed following standard treatment alone (Fig. 5G). Additionally, although the expression of ISGs in the entire LDG cluster showed no significant decrease relative to baseline after belimumab treatment (Fig. 3A), it was significantly downregulated in LDG-SC1 (Fig. 5H) and SC2 (Fig. 5I) in this group of patients.

The expression of the cell surface neutrophil maturation marker FCGR3B (CD16b) was also detected in both LDG-SC1 and LDG-SC2 (Fig. 5B-C). CD16b+ LDGs have been reported to upregulate neutrophil activation and the IFN-I pathway (22). In our dataset, the expression of CD16b was significantly decreased in LDGs after belimumab + ST but not after ST alone (Fig. 5J). These results suggested that belimumab might inhibit the IFN-I signalling pathway in patients with SLE by targeting $CD16b^+$ LDG-SCs in which ISG expression is elevated.

Belimumab affected the expression of TACI, BAFF-R, BCMA in circulating immune cell subsets

Belimumab has been reported to inhibit

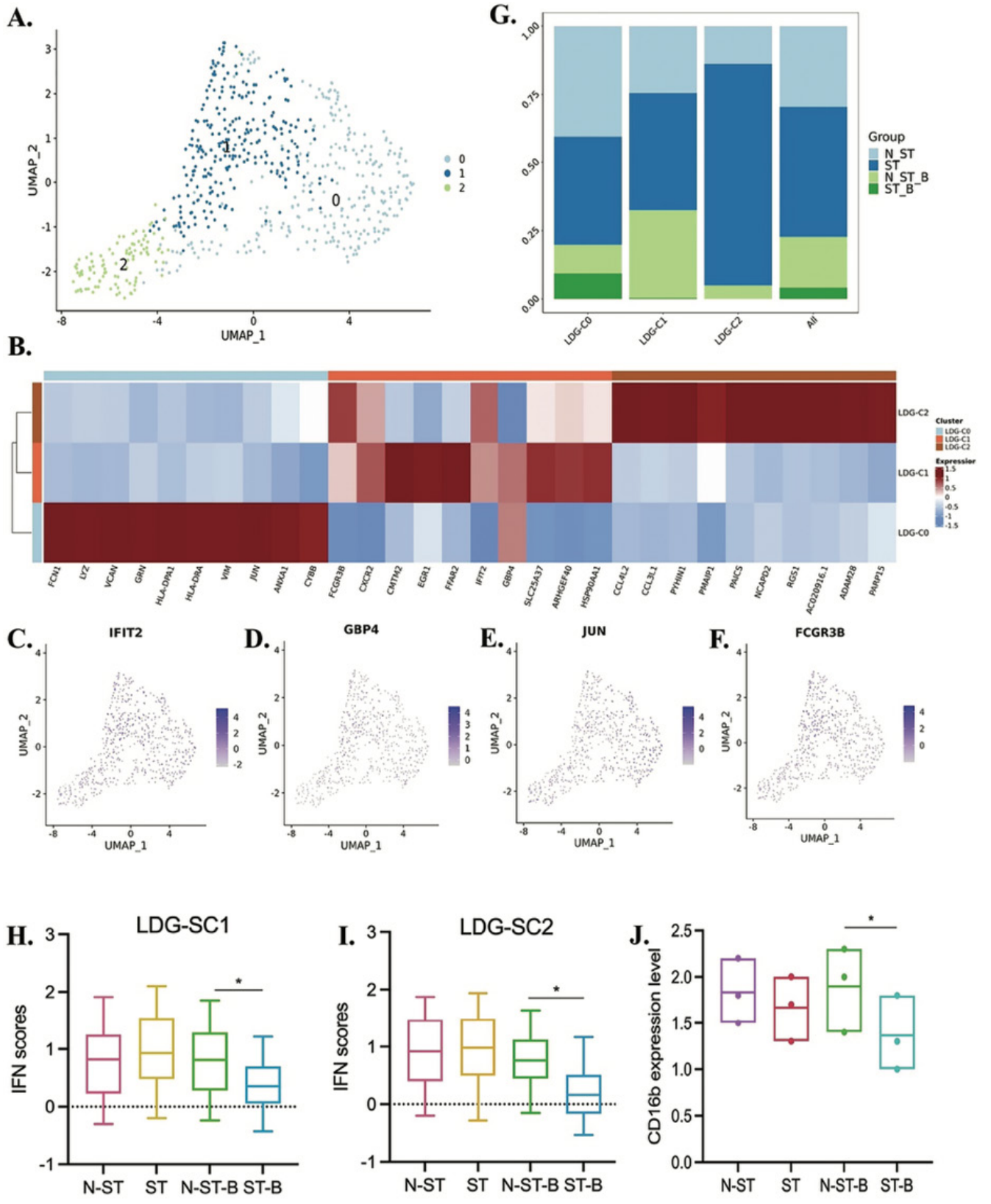

Fig. 5. Belimumab affected the IFN-I pathway in low-density granulocyte (LDG) subclusters (SCs).
(**A**) Alterations in the proportions of LDG clusters after standard treatment or standard treatment + belimumab. A uniform manifold approximation and projection (UMAP) plot representing LDG subclusters (LDG-SCs, n=3) from patients with systemic lupus erythematosus (SLE). (**B**) Heatmap displaying the expression of the genes defining each of the mono-SCs. (**C**-**F**) UMAP plots relating to the expression values for the *IFIT2* (**C**), *GBP4* (**D**), *JUN* (**E**), and *FCGR3B* (**F**) genes. (**G**) A bar plot highlighting cell abundances across the LDG-SCs for all the groups. (**H, I**) IFN scores for LDG-SC1 (**H**) and LDG-SC2 (**I**) between the different groups. (**J**) The expression of CD16b in LDGs from individual samples from the different groups (*p<0.05).

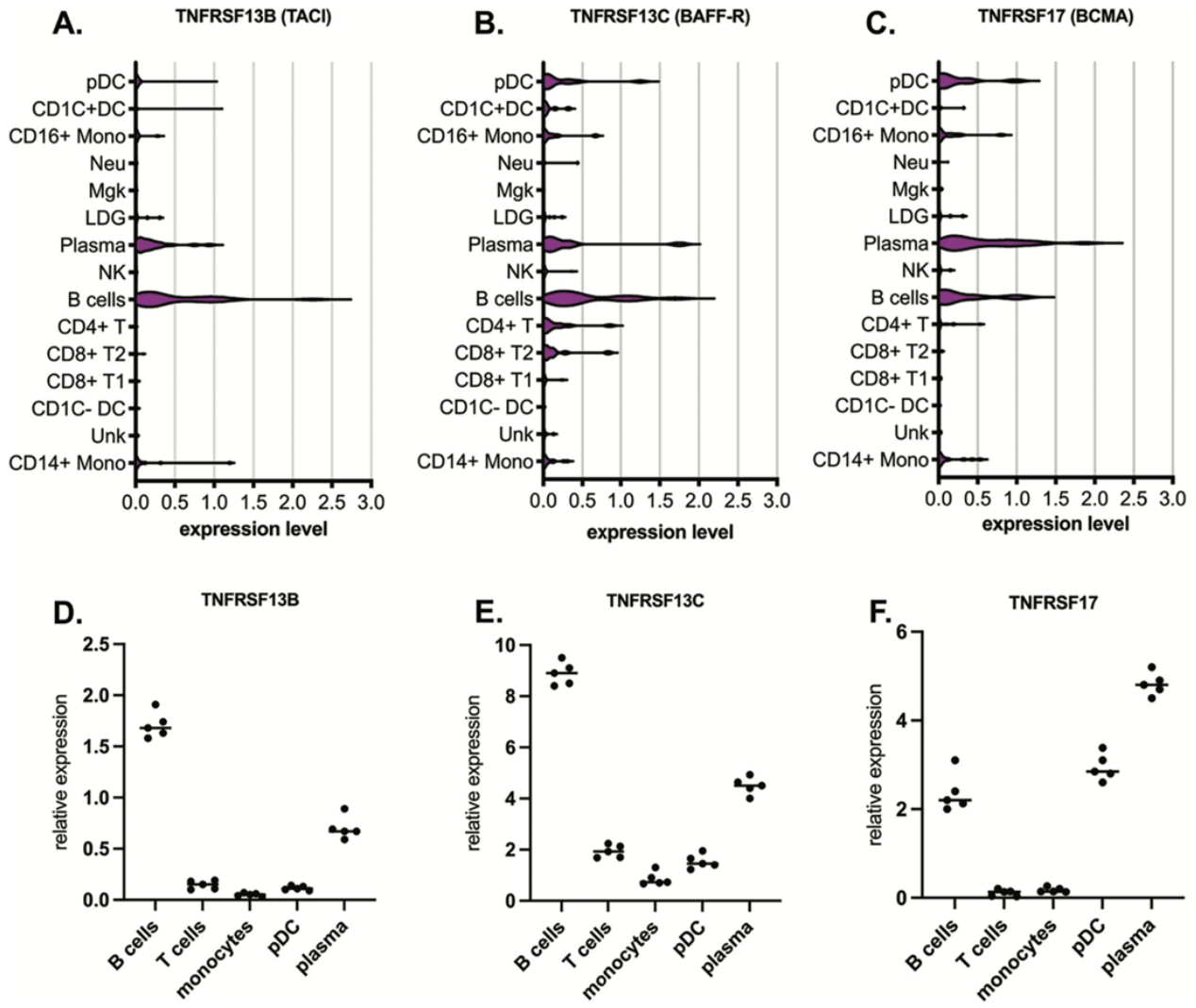

Fig. 6. Belimumab affected the expression of TACI, BAFF-R, BCMA in circulating immune cell subsets.
The expression of three BAFF receptors (BCMA, BAFF-R, and TACI) in patients with systemic lupus erythematosus (SLE). (**A**-**C**) The distribution of TACI (**A**) BAFF-R (**B**) and BCMA (**C**) transcripts in the single-cell RNA sequencing dataset. (**D**-**F**) RT-qPCR analysis of the expression of TACI (**D**), BAFF-R (**E**), and BCMA (**F**) in sorted immune cell subsets from SLE patients.

the binding of BAFF to its receptors TACI, BCMA, and BAFF-R. Accordingly, we next evaluated the expression of the three receptors in immune cell populations from the peripheral blood of SLE patients. The scRNA-seq data revealed that *TNFRSF13B* (TACI) (Fig. 6A) and *TNFRSF13C* (BAFF-R) (Fig. 6B) were most prominently expressed in B cells, while *TNFRSF17* (BCMA) (Fig. 6C) was mainly expressed in plasma cells. Importantly, circulating SLE pDCs expressed both *TNFRSF13C* and *TNFRSF17*, with *TNFRSF17* expression in pDCs being comparable to that in B cells. *TNFRSF13C* expression was also detected in monocytes and T cells, albeit at a substantially lower level than in B cells. These results were further confirmed by RT-qPCR analysis in selected cell clusters from PBMCs of five subjects with active SLE. The expression of TNFRSF13B (Fig. 6D), TNFRSF13C (Fig. 6E), and TNFRSF17 (Fig. 6F) in B cells or plasma cells were prominently increased in belimumab treatment group.

Discussion

BAFF is a cytokine essential for B-cell development and function. Studies have suggested that belimumab, which targets BAFF, exerts rapid effects on immature B cells (23, 24). These cells are highly sensitive to the pleiotropic actions of IFN, and IFN-I can induce feed-forward BAFF production (25-27). In this study, we used scRNA-seq to uncover the early effects of IFN signalling and reveal the IFN-associated cell subsets during treatment with belimumab in SLE patients. Major leucocyte clusters were identified and the expression of ISGs in monocyte and LDG-SCs was found to be regulated by belimumab-mediated BAFF inhibition in SLE patients. Our results agree with previous studies that used scRNA-seq of PBMCs to identify cell subpopulations that contribute to the IFN-I signature in SLE (28, 29). Importantly, we propose a potential novel mechanism underlying the therapeutic effects of belimumab in SLE. After 8 weeks of belimumab + ST, the proportions of subpopulations of monocytes and LDGs with high ISG expression were significantly decreased compared

with ST alone. Although the treatment duration was short, it is noteworthy that immunological responses to belimumab preceded overt clinical improvements (30). This alteration in the IFN-I signature in immune cell populations may prove a useful therapeutic and disease-monitoring tool for SLE. Our data support the use of add-on belimumab versus standard care alone in patients showing an elevated IFN signature. However, whether such changes are limited to the short term after therapy commencement requires further investigation.

The presence of non-specific immune cell, such as monocytes and granulocytes, is indicative of an active immune response to external or internal antigens. Human peripheral monocytes can be broadly categorised into three subsets, nonclassical ($CD14^{+}$ $CD16^{++}$), intermediate ($CD14^{++}CD16^{+}$) and classical monocytes ($CD14^{++}CD16^{-}$). Some studies on monocyte subpopulations have suggested that the frequency of $CD16^{+}$ subsets is elevated in SLE (21, 31). $CD16^{+}$ monocytes are considered pro-inflammatory and play a critical role in promoting $CD4^{+}$ T-cell polarisation into a Th17 phenotype and the differentiation of B-cell responses in SLE. Our study showed that belimumab treatment can inhibit the expansion of $CD16^{+}$ monocytes in SLE patients. The increased proportion of $CD16^{+}$ monocytes in the ST group is suggestive of insufficient control of the inflammatory immune response by the respective immunosuppressive agents. In contrast, the targeted blocking of B-cell-related cytokines elicited an obvious effect on reducing the inflammatory response, in agreement with recent reports indicating that belimumab can be applied for the treatment of lupus nephritis (32, 33).

LDGs display an enhanced ability to form proinflammatory neutrophil extracellular traps (NETs) in the context of SLE (34). After quality control, only 630 LDGs were identified in our scRNA-seq data. This might be attributed to the potent inhibition of neutrophils in lupus patients induced by immunosuppressive drugs. We found that ISG activity was prominent in LDGs and belimumab treatment reduced the IFN score of LDG-SC1 and LDG-SC2, both of which expressed FCGR3B (CD16b). Furthermore, previous studies have suggested that the IFN immune response in LDGs is associated with increased aggressiveness and organ damage in SLE patients (22, 35). Given that BAFF is highly expressed by LDGs in the context of SLE (36), therapy with belimumab, which targets BAFF, is expected to reduce neutrophil-driven inflammation in SLE patients. Moreover, clinical study reported that combination therapy with rituximab and belimumab resulted in the abrogation of excessive NET production in SLE (37). These observations imply that neutralising BAFF can indirectly reduce IFN-I responses by inhibiting NET formation in SLE LDGs, a process that is crucial for IFN-I production (38).

BAFF plays a crucial role not only in B cells but also in other immune cells, such as T cells and monocytes (6, 10). In this study, *BCMA* and *BAFF-R* mRNA expression was detected in human pDCs derived from patients with SLE. This suggests that belimumab may directly regulate the function of pDCs, which are the major source of IFN-I induced by antibody-nucleic acid immune complexes in SLE. In healthy, the BCMA receptor was reported to be displayed on the surface of circulating pDCs after TLR7/8 or TLR9 activation (39). Further investigation is warranted to elucidate whether the BAFF receptors are expressed on the surface of pDCs in SLE, as well as to better understand their roles in the pathology of this disease.

While our study uncovered important novel alterations in the IFN signature during belimumab treatment, it nonetheless had several limitations. First, the sample size of the enrolled lupus patients was limited. A clinical study with a large sample size and long-term follow-up is required to further validate our findings. Second, some blood cell types, such as LDGs and plasma cells, may have been inefficiently collected or preferentially depleted during the scRNA-seq process. Third, from a clinical perspective, predictors for different patient subgroups need to be identified to facilitate an individualised management approach. However, we were unable to analyse subgroups of patients with high baseline BAFF or IFN-I mRNA levels. Despite these limitations, our study, which was based on short-term observation, provided preliminary data that is expected to drive more conclusive testing in the future.

In summary, the results of this study suggested that belimumab treatment can modulate the IFN-I signature in specific types of immune cells derived from the peripheral blood of patients with SLE in the short term. Notably, CD16+ monocytes and LDGs may be the key contributors to ISG expression, and belimumab may influence this process. Due to sample size limitations, additional studies involving larger sample sizes and longer follow-up times are warranted to confirm these findings.

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