Behçet’s disease (BD) is a chronic vasculitis characterized by systemic immune aberrations. However, a comprehensive understanding of immune disturbances in BD and how they contribute to BD pathogenesis is lacking. Here, we performed single-cell and bulk RNA sequencing to profile peripheral blood mononuclear cells (PBMCs) and isolated monocytes from BD patients and healthy donors. We observed prominent expansion and transcriptional changes in monocytes in PBMCs from BD patients. Deciphering the monocyte heterogeneity revealed the accumulation of C1q-high (C1qhi) monocytes in BD. Pseudotime inference indicated that BD monocytes markedly shifted their differentiation toward inflammation-accompanied and C1qhi monocyte–ended trajectory. Further experiments showed that C1qhi monocytes enhanced phagocytosis and proinflammatory cytokine secretion, and multiplatform analyses revealed the significant clinical relevance of this subtype. Mechanistically, C1qhi monocytes were induced by activated interferon-γ (IFN-γ) signaling in BD patients and were decreased by tofacitinib treatment. Our study illustrates the BD immune landscape and the unrecognized contribution of C1qhi monocytes to BD hyperinflammation, showing their potential as therapeutic targets and clinical assessment indexes.

Behçet’s disease | single-cell analysis | monocytes | interferon

Behçet’s disease (BD) is a systemic inflammatory disorder affecting blood vessels and commonly manifests as recurrent oral/genital ulceration and skin lesions, while patients with exacerbated BD exhibit multiorgan involvement such as uveitis and gastrointestinal, neurological, vascular, and cardiac symptoms, which cause significant morbidity and mortality (1–3). BD displays geographical variations in prevalence, with a higher incidence (20 to ~602 cases per 100,000 population) in Silk Road countries spanning from China in the east to Turkey in the Mediterranean area (2–6). The atypical symptoms and regional differences in disease prevalence pose diagnostic and therapeutic challenges for clinicians. Thus, the identification of laboratory indices to improve empirical judgment and BD therapies is needed.

Accumulating evidence has shown that immunological abnormalities are pivotal in BD development, although genetic variants and environmental stimuli are also important triggers (7). Patients with BD exhibit aberrant and excessive activation of both innate and adaptive immunity (corresponding to the features of autoimmune or autoimmune diseases) (7, 8), including the overproduction of proinflammatory cytokines (interferon-γ [IFN-γ], interleukin 6 [IL-6], and tumor necrosis factor α [TNF-α]) (9) and skewed T-helper (Th) 1 and Th17 cell activation (10, 11). Intense efforts into understanding BD pathogenesis have focused on specific immune cells based on select cell-surface markers (12–14). However, a comprehensive and unbiased depiction of immune disturbances in BD remains to be elucidated.

In previous BD studies, the transcriptomic profiling at the bulk level lacked the resolution to capture cellular heterogeneity and was limited in the ability to identify the pathogenetic contribution of certain cell subtypes (15–17). Recently, single-cell RNA sequencing (scRNA-seq) technology has shown unprecedented value in discovering pathological immune changes at higher resolution (18–23). However, the molecular characteristics of diverse immune cell subsets associated with BD at single-cell resolution and how these features contribute to exaggerated inflammation in BD patients have not been previously described.

To address these questions in an unbiased manner, we applied scRNA-seq to first delineate the immune landscape in peripheral blood mononuclear cells (PBMCs) that usually serve as an attractive source for understanding the pathogenesis of diverse diseases with abnormal immunophenotypes (24–26). Monocytes with significant

**Significance**

Behçet’s disease (BD) is a systemic vasculitis, and its pathogenesis is elusive. Limited understanding of the immune disturbances in BD hindered the identification of therapeutic targets. Here, we performed single-cell and bulk RNA sequencing to reveal the comprehensive landscape of cellular and molecular changes in BD blood. We observed the mobilization of monocytes, especially a monocyte subset (C1q-high monocytes) in BD. Further assays revealed the proinflammatory features and clinical relevance of this subset. Activated interferon-γ (IFN-γ) signaling in BD patients induced C1q-high monocyte expansion, which was recovered by tofacitinib treatment. Our findings provide comprehensive understanding of BD immunopathogenesis, highlighting the proinflammatory contribution and therapeutic potential of C1q-high monocytes.
expansion and transcriptional changes were observed in BD. Further investigations demonstrated that the unappreciated C1q-high (C1qhi) monocyte subtype exhibited proinflammatory characteristics and significant clinical relevance in BD. Overall, our study offers a comprehensive view of immune dysfunction in BD and highlights the proinflammatory contribution of C1qhi monocytes to BD immunopathogenesis, which might facilitate the development of clinical assessments and targeted therapies for BD.

Results

The Single-Cell Landscape of Peripheral Mononuclear Cells in BD. We performed droplet-based scRNA-seq (10X Genomics) to delineate the immune landscape of PBMCs from four treatment-naive BD patients and four healthy controls (HCs) (Fig. 1A and Dataset S1). A total of 36,190 high-quality cells of 41,889 cells were retained for downstream analysis after removing cells with a low sequence depth and a high ratio of mitochondrial genes (SI Appendix, Fig. S1A); the analyzed cells included 17,678 (48.8%) from BD patients and 18,512 (51.2%) from HCs, and an average of 1,096 genes per cell was monitored (SI Appendix, Fig. S1B–D). After batch effect correction, dimension reduction, and graph-based clustering, we identified 20 distinctive clusters (Fig. 1B) within five major cell lineages, including myeloid cells (CD3D+), T cells (CD3D+), natural killer (NK) cells (KLRF1+), B cells (CD79A+), and nonimmune cell lineages (PPBP+) (Fig. 1C). As expected, unsupervised hierarchical clustering of all cell clusters confirmed the major cell lineages (Fig. 1D).

The high sensitivity of scRNA-seq allowed us to further map these clusters to immune cell subsets. Specifically, we identified four myeloid cell subtypes according to the expression of canonical markers and cell type–specific gene sets (27) (Fig. 1E and SI Appendix, S1 E and F and Dataset S2), including CD14+ monocytes (LYZ CD14+), CD16+ monocytes (LYZ FCGR3A+), conventional dendritic cells (cDCs; CD1C+CLEC10A+), and plasmacytoid dendritic cells (pDCs; LILR4A+CLEC4C+). We also identified nine T cell subtypes, namely naive CD4 T cells (CD4 T naive; CD3D+CCR7+), memory CD4 T cells (CD4 T memory; CD3D+CD40LG+), IFN-related CD4 T cells (CD4 T IFNrelated; CD3D+ISG15+), regulatory T cells (Tregs; CD3D FOXP3+), innate-like T cells (innate lymphoid cells; CD3D+CD127+KLRG1+SLC4A10+), memory CD8 T cells (CD8 T memory; CD8A+GZMK+), effector CD8 T cells (CD8 T effector; CD8A+GZMB+), and proliferating T cells (proliferating T; CD3D*MKI67+ (SI Appendix, Fig. S1H), which were confirmed by evaluating T cell signature gene sets (27) (SI Appendix, Fig. S1G). In addition, other subpopulations were annotated mainly according to unique marker genes, including resting NK cells/CD56NCAM1+NCAM1+CD16+ NK (NCAM1hiCD16+ KLRF1+ GZMK), active NK cells/CD56dimCD16+ NK (NCAM1dimCD16+ KLRF1+GZMB), naive B cells (CD79A+IL4R), memory B cells (CD79A+CD27), plasma B cells (CD79A+MZB1), megakaryocytes (PPBP+), and erythrocytes (HBAT) (Fig. 1E and SI Appendix, Fig. S1H). All clusters contained cells from multiple BD patients, suggesting the absence of significant batch effects (Fig. 1F and SI Appendix, Fig. S1J). Thus, we delineated the peripheral immune landscape in BD at single-cell resolution.

Monocytes Contribute to Systemic Immune Aberrations in BD. Aiming to decipher the changes in immune cell composition in BD, we performed integrative analyses of scRNA-seq data with bulk RNA-seq data (9 BD patients and 10 HCs) to increase patient numbers. Principal component analysis of bulk RNA-seq data using all quantified genes revealed that the first principal component clearly distinguished BD patients from HCs (SI Appendix, Fig. S2A), indicating dramatic differences in the genome-wide profiles of BD patients compared with HCs. We then identified 1,514 up-regulated and 325 down-regulated differentially expressed genes (DEGs) in BD from bulk RNA-seq data (Fig. 2A). Upon mapping these DEGs to cell types identified in scRNA-seq data, we found that the up-regulated DEGs were relatively enriched in monocytes and DCs (Fig. 2B), while the down-regulated genes were enriched in B cells (Fig. 2C). However, the fold changes in these DEGs between BD patients and HCs did not reveal a pronounced enrichment in specific cell types (SI Appendix, Fig. S2B). This result implies that BD-associated variations in the bulk transcriptome are more likely to be impacted by changes in cellular composition than by intracellular changes. We next deconvoluted the immune cell subtypes in bulk RNA-seq data by CIBERSORT (28) with the LM22 signature matrix, which distinguishes human hematopoietic populations isolated from peripheral blood (28). Consistently, we found a higher proportion of monocytes in BD patients than in HCs (Fig. 2D), which was further confirmed by complete blood count data (23.3 ± 10.1% versus 12.4 ± 2.8%, P < 0.0001; Fig. 2E) and scRNA-seq data (SI Appendix, Fig. S2C), suggesting an aberrant role of monocytes in BD. Additionally, smaller memory B cell populations were observed in BD patients than in HCs (Fig. 2F), as previously reported (29).

Next, we investigated intracellular biological differences associated with BD development in the major cell lineages using scRNA-seq data. We identified DEGs that distinguished BD from HC in individual cell lineages and determined the enriched biological pathways. The bacterial and viral infection pathways were enriched in most lineages (SI Appendix, Fig. S2D), supporting the hypothesis that infectious agents trigger BD pathogenesis (30, 31). In T cells, Th1 cell differentiation was among the top enriched pathways (SI Appendix, Fig. S2D), suggesting Th1-mediated hyperactivity in BD (32). In monocytes, the top pathways were the IFNγ response, processing and presentation of exogenous antigens, and neutrophil activation (Fig. 2F and G and Dataset S3). Moreover, the up-regulated DEGs in monocytes from scRNA-seq data were shared with the DEGs from the bulk RNA-seq data (SI Appendix, Fig. S2E), further confirming the significant contribution of monocytes to the global transcriptome aberrations in BD. Taken together, the integrated analysis of bulk RNA-seq data systematically identified BD-associated immune disturbances and indicated the prominent mobilization of monocytes in BD.

Identification of Monocyte Heterogeneity. To increase cell-level resolution and dissect monocyte heterogeneity, we performed scRNA-seq of magnetic bead–sorted monocytes (CD14+) from PBMCs of four BD patients and four HCs. We retained 39,385 monocytes that passed rigorous filtering, including 17,321 cells (44%) from HCs and 22,064 cells (56%) from BD patients. By graph-based clustering, we captured the profiles of eight clusters (Fig. 3A and Dataset S4), which were not confounded by either a specific patient or condition (SI Appendix, Fig. S3 A and B).

The heterogeneity of monocytes corresponds to diverse functional specialization (33). We then compared the functional phenotypes of all eight subtypes by assessing the highly expressed genes and corresponding enriched pathways (Fig. 3 B and C). Five of the eight monocyte subtypes maintained higher levels of
CD14 (Fig. 3C). Specifically, VIM-high monocytes (VIM Monos) overexpressed S100A family genes (S100A8, S100A9, and S100A12), which are markers of human myeloid-derived suppressor cells (34). Although the expression profile of SOD2-high monocytes (SOD2 Monos) resembled that of VIM Monos (SI Appendix, Fig. S3C), SOD2 Monos were enriched in the
response to oxidative stress and overexpressed redox-related genes (SOD2, CYBA, and NAMPT) that are associated with impaired fibrinogen function in BD patients (35). EIF5A-high monocytes (EIF5A Monos) involved cell proliferation genes (EIF5A and C1orf56); ISG15-high monocytes (ISG15 Monos) showed preferential expression of IFN-inducible genes (ISG15, MX1, and MX2); and major histocompatibility complex (MHC)-II–high monocytes (MHC-II Monos) were enriched for genes in antigen presentation pathways, such as those encoding MHC class II chains (HLA-DRA, HLA-DQA1, HLA-DPB1, and CD74). Among the three subtypes with lower CD14 expression (Fig. 3C), CD16-high monocytes (CD16 Monos) presented nonclassical monocyte markers (FCGR3A, MS4A7, and CX3CR1), and monocyte-derived DCs (MoDCs) showed overexpression of DC...
markers (CLEC10A, FCER1A, CST3, and CD74) with enrichment of antigen presentation pathways and the DC-specific gene signature (Fig. 3 B and C and SI Appendix, Fig. S3D). C1q\textsuperscript{hi} monocytes (C1Q Monos) highly expressed components of complement 1q (C1q genes, including C1QA, C1QB, and C1QC) and macrophage markers (CD68) (Fig. 3C) and also exhibited a macrophage-like phenotype (SI Appendix, Fig. S3D).

We next investigated BD-associated differences in monocyte composition. Comparing the relative cell proportions in the BD and HC groups, we observed a notable increase in C1Q Monos in BD patients and a significant decrease in MoDCs (Fig. 3D). Using flow cytometry, we observed the existence of this subtype and validated that the C1q\textsuperscript{hi} monocyte population (C1Q Monos in scRNA-seq data) was increased in active BD patients (1.28 ± 0.99% versus 0.73 ± 0.44%, \( P = 0.0024 \); Fig. 3 E and F). Altogether, analysis of the scRNA-seq data for sorted monocytes revealed the heterogeneity of circulating monocytes and the corresponding diversification of monocyte functions and significantly increased C1q\textsuperscript{hi} monocytes in BD patients.

**Differentiation Trajectories Toward C1q\textsuperscript{hi} Monocytes Are Accompanied by Inflammatory Pathways.** To explore the transitional relationships across monocyte subtypes, we determined the pseudotemporal order and reconstructed the differentiation
trajectory using a diffusion map (36), TSCAN (37), and Sling-shot algorithms (38). The pseudotime trajectory axes branched from MHC-II Monos to two termini: fate 1, C1Q Monos, and fate 2, MoDCs (Fig. 4A and B). These two fates corresponded to the roles of monocytes in replenishing macrophages and DCs (39) and were confirmed by Monocle 3 (40) (SI Appendix, Fig. S4A). Interestingly, a significantly imbalanced distribution of two conditions appeared along with the two fates, with BD monocytes aggregating at the end of fate 1 but more HC monocytes accumulating at the end of fate 2 (Fig. 4C). This result corresponded with the increased C1Q Monos and decreased MoDCs signatures in BD (Fig. 3D). The remarkable shift in monocyte differentiation toward C1Q Monos, but not MoDCs, suggests the role of C1Q Monos in modulating exaggerated inflammation in BD.

Next, we investigated the correlated gene expression profiles and pathways underlying the two differentiation trajectories (Dataset S5). We observed the gradual up-regulation of genes encoding inflammatory cytokine-related proteins (ISG15, TNRFSF18, and $\alpha$100A11) and complement components (C1q genes) along fate 1 (Fig. 4D). In contrast, fate 2 showed variations in antigen presentation genes, including MHC-II genes (HLA-DR, HLA-DP, and HLA-DQ), the Fc fragment of the immunoglobulin E receptor (FCER1A and FCER2B), and DC markers (CLEC10A, CD1C, and CD74) (Fig. 4E). Pathway enrichment analyses identified fate 1 was in conjunction with inflammation-related pathways (41-43), such as chemokine signaling pathway, nucleotide oligomerization domain (NOD)-like receptor signaling pathway, and NK cell-mediated cytolysis, while fate 2 was associated with antigen presentation-related pathways. These results indicated functional divergence of cells adopting the two fates. As expected, phagocytosis and antigen presentation were enhanced along fate 1 and fate 2, respectively (SI Appendix, Fig. S4 B and C). In addition, the expression of transcription factors (TFs) engaged in the monocyte-to-macrophage transition (RHOC, NR4A1, and MAFB) gradually increased in fate 1, which ended with the macrophage-like subtypes (C1Q Monos), but not in fate 2, which concluded with the MoDCs subtype (Fig. 4G and SI Appendix, Fig. S4D).

Altogether, monocytes in BD patients preferentially differentiate toward a C1Q Monos-ended and inflammation-accompanied trajectory, which suggests the proinflammatory characteristics of C1Q Monos.

C1Q Monos Contribute to Hyperinflammation in BD. To test whether C1Q Monos manifest proinflammatory properties, we analyzed three key inflammation-regulatory capacities of monocytes, including phagocytosis, antigen presentation, and cytokine secretion. C1Q Monos indicated greater functional capacity than other subtypes (Fig. 5A), as evidenced by the overexpression of BD-associated proinflammatory cytokines (TNF and IL6) (47, 44), FcR activators (LYN and HCK), recruits of cytoskeleton remodelers (PIK3CG, WASP2, and VASP), and MHC-II components (SI Appendix, Fig. S5 A-C).

Next, we examined the phagocytic capabilities by incubating monocytes with fluorescein-labeled dextran. BD C1qhi monocytes engulfed more dextran than CD16+ monocytes (Δmean fluorescence intensity [MFI]: 832.8 ± 175.4 versus 567.8 ± 43.5, P = 0.0175) and CD16- monocytes (ΔMFI: 832.8 ± 175.4 versus 448 ± 84.6, P = 0.0045; Fig. 5 B and C and SI Appendix, Fig. S5D). We further detected proinflammatory cytokine production in lipopolysaccharide (LPS)-stimulated monocytes and observed that BD C1qhi monocytes produced significantly higher levels of IL-6 and TNF-α than CD16+ monocytes (IL-6: 5.1 ± 2.8% versus 2.5 ± 0.9%, P = 0.0362; TNF-α: 58.5 ± 3.0% versus 47.1 ± 7.1%, P = 0.0034) or CD16- monocytes (IL-6: 5.1 ± 2.8% versus 1.2 ± 0.5%, P = 0.0191; TNF-α: 58.5 ± 3.0% versus 31.4 ± 4.4%, P < 0.0001; Fig. 5 D-G). Collectively, our functional assays confirmed the proinflammatory characteristics of C1qhi monocytes, providing insights into the pathogenicity of C1qhi monocytes in the hyperinflammatory responses of BD patients.

Activated IFN-γ Signaling Stimulates the Expansion of C1qhi Monocytes in BD. To understand the underlying mechanisms of C1qhi monocyte expansion in BD, we first analyzed DEGs in C1qhi monocytes between BD and HC and identified 254 up-regulated genes and 115 down-regulated genes in BD (SI Appendix, Fig. S6A). The up-regulated genes were predominantly enriched in pathways of response to IFN-γ (Fig. 6A), corresponding to the global overexpression of IFN-γ-inducible genes in C1qhi monocytes (SI Appendix, Fig. S6A) and the DEG enrichment in total monocytes (Fig. 2G). Additionally, the overexpression of these IFN-induced genes (ISG15, LYE6, IFITM2, and IFITM3) was accompanied by preferential differentiation into C1qhi monocytes (Fig. 4D). Next, we used SCENIC tools (45) to predict which TFs modulate these DEGs and noted the marked enrichment of TFs that regulate IFN-γ response pathways, including STAT1 and IRF1 (Fig. 6B and SI Appendix, Fig. S6B); the expression of these TFs was also elevated in C1qhi monocytes from BD patients (SI Appendix, Fig. S6C). STAT1 phosphorylation was validated to be significantly increased in C1qhi monocytes from BD patients (normalized MFI: 1.5 ± 0.7 versus 0.9 ± 0.3, P = 0.013; Fig. 6C).

The enriched IFN-related pathways in BD C1qhi monocytes inspired us to ask whether IFNs can stimulate C1qhi monocytes. We found that IFN-γ treatment significantly increased the expression of C1q genes in HC monocytes (fold change: 2.0 ± 0.9 for C1QA, P = 0.028; 29.2 ± 11.5 for C1QB, P < 0.0001; 2.2 ± 1.2 for C1QC, P = 0.032; Fig. 6D) and increased the proportion of C1qhi monocytes (3.0 ± 2.1% versus 0.8 ± 0.5%, P = 0.013; Fig. 6 E and F), which was significantly higher than IFN-α or IFN-β (SI Appendix, Fig. S6 D-F).

Meanwhile, we observed markedly increased IFN-γ concentrations in serum from BD patients (14.6 ± 14.8 pg/mL versus 4.012 ± 3.2 pg/mL, P < 0.0001; SI Appendix, Fig. S6G). By examining the main IFN-γ-producing cells, we found a significant increase in Th1 cells (IFN-γ-positive CD4 cells) among CD4+ T cells (12.9 ± 4.7% versus 8.15 ± 2.6%, P = 0.038; SI Appendix, Fig. S6 H and J). To gain insights into the regulatory relationships among these cell clusters, we inferred putative interactions between C1qhi monocytes and T/NK cells (SI Appendix, Fig. S7A and Dataset S6). Incoming interactions from T/NK cells to C1qhi monocytes were increased mainly in CD4+ T cells from BD patients but were reduced in CD8+ T cells and NK cells, in line with the increased IFN-γ production by CD4+ T cells. Additionally, the increased number of outgoing interactions from C1qhi monocytes to T/NK cells indicates intense signalings were sent from C1qhi monocytes in BD patients. Proinflammatory characteristics were also shown in BD-associated interactions, including CD400-CD40LG (46), HLA-DPB1-TNFFS13B (47), CXL10-CXCR3 (48), and TNFFRSF10B-TNFFS10 (49) (SI Appendix, Fig. 57 B and C and Dataset S6). These analyses reveal frequent crosstalk between C1qhi monocytes and CD4+ T cells in BD patients.

IFN-γ is a major effector in the pathogenesis of numerous inflammatory and autoimmune diseases (50, 51). We evaluated whether C1qhi monocytes are also present in patients with...
cancer or other IFN-γ–related immune diseases. Through an integrative analysis, we observed that the C1qhi monocytes in BD showed similar transcriptomic patterns to the C1QA monocytes found in rheumatoid arthritis (SDY998) (52) (SI Appendix, Fig. S7D). We also observed C1qhi monocytes in other immune diseases, such as systemic lupus erythematosus (Gene Expression Omnibus [GEO] database accession No. GSE135779) (53), and Kawasaki disease (GSE168732) (54) (SI Appendix, Fig. S7D), but not in blood cancers, including acute myeloid leukemia (GSE116256) (55), acute lymphocytic leukemia (GSE132509) (56), and chronic lymphocytic leukemia patients (GSE111014) (57) (SI Appendix, Fig. S7E and Dataset S7). Altogether, our data suggest the unrecognized mechanism by which IFN-γ promotes the generation of C1qhi monocytes in
**Fig. 5.** C1qhi monocytes exhibited proinflammatory properties. (A) Violin plots showing the average expression levels of monocyte functional gene sets (SI Appendix, Materials and Methods) among all monocyte subtypes (colors). Top: BD patients; Bottom, HCs. Groupwise P values were calculated via the Kruskal-Wallis test, and pairwise P values were calculated via the Wilcoxon test. (B and C) Representative histograms (B) and summary (C) of the phagocytosis capability of C1qhi monocytes, non-C1qhiCD16+ monocytes, and non-C1qhiCD16− monocytes from BD patients (n = 5) and HCs (n = 5). The data are summarized as the mean ± SD. (D–G) Representative histograms (Top) and summary (Bottom) of the flow cytometry analysis results showing the significantly higher production of IL-6 (n = 5 in BD and n = 9 in HC; D and E) and TNF-α (n = 5 in BD and n = 9 in HC; F and G) in C1qhi monocytes than in non-C1qhiCD16+ monocytes and non-C1qhiCD16− monocytes after LPS stimulation. The data are summarized as the mean ± SD. Paired t tests (C, E, and G) were applied. TRITC-dextran, tetramethylrhodamine isothiocyanate-dextran; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. ns, not significant.
BD, and this mechanism may also be involved in the pathogenesis of other immune diseases.

**C1qhi Monocytes Distinguish BD Patients From Healthy Donors and Respond to Treatment.** To explore the potential clinical value of C1q Monos, we mined the predictive power of highly expressed genes in C1q Monos in our in-house cohort (n = 19) and three published cohorts with active BD patients (GSE17114, n = 29; GSE165254, n = 25; and GSE70403, n = 58). The top five expressed genes in C1q Monos were found to significantly explain variance and predict BD patients with an area under the curve above 0.7 in the three cohorts (Fig. 7A). Consistently, these five genes showed significantly higher relative expressions than randomly selected genes in the HC-corrected gene profiles of BD patients in the GSE70403 cohort (SI Appendix, Fig. S8A). Among the top five genes, C1q genes (C1QA, C1QB, and C1QC) were significantly up-regulated in the bulk RNA-seq analysis of PBMCs (SI Appendix, Fig. S8B), and C1q protein levels were also markedly increased in serum from BD patients (108.9 ± 18.4 μg/mL versus 82.0 ± 26.8 μg/mL, P = 0.03; SI Appendix, Fig. S8C). These results reveal C1Q Monos could distinguish active BD patients from HCs in diverse cohorts.

We investigated BD-associated genes identified by genome-wide association studies across monocyte subtypes and found that C1Q Monos had the highest average expression of these genes (SI Appendix, Fig. S8D). Furthermore, C1qhi monocytes were positively correlated with the erythrocyte sedimentation rate (a clinical diagnostic parameter measuring the degree of BD inflammation) (r = 0.46, P = 0.004; SI Appendix, Fig. S8E) and showed higher proportions in BD patients with high disease activity scores (estimated by Behçet’s Disease Current Activity Form [BDCAF] scores) (Fig. 7B). In brief, these results reveal the significant association of C1qhi monocytes with disease activity, indicating their potential as clinical parameters to identify BD patients in the active inflammation stage.

Furthermore, we explored whether C1qhi monocytes could respond to drug treatment in BD. The immunosuppressive therapies significantly decreased C1qhi monocytes within the same patient after treatment (1.4 ± 1.2% versus 0.5 ± 0.4%,...
Having observed the prominent activation of STAT1 and canonical pathway that IFN- γ and activator of transcription (STAT) pathway signaling is a potential of C1qhi monocytes in monitoring treatment efficacy and indicate that targeting C1qhi monocytes could be an effective strategy for BD therapies.

### Discussion

BD shares common features with autoinflammatory and autoimmune diseases (2, 5, 7). Our findings focused on revealing the unexplored immune landscape in BD at single-cell resolution, especially the markedly increased monocyte population. Among monocyte subtypes, the increased C1qhi monocytes manifested proinflammatory features and significant clinical relevance. This subtype was induced by IFN-γ in BD serum but was recovered by tofacitinib treatment. Briefly, our study delineates a single-cell view of circulating blood in BD patients,
increasing the understanding of how certain subtypes contribute to BD hyperinflammatory status and revealing potential targets for BD therapies and clinical assessment.

Monocytes serve as a bridge between innate and adaptive immunity owing to their versatile functions, including antigen presentation, phagocytosis, and cytokine secretion (59). Impaired monocyte function is emerging in multiple autoimmune and autoimmune diseases (60–64). Our study found increased monocytes in BD blood, which may contribute to aberrant monocyte infiltration in the BD lesions, such as intestinal ulcers, blood vessels and the brain (65–67). Monocytes are a heterogeneous population of cells with functional variation (33), our previous study revealed the dysfunction of monocyte subsets in BD (50). Here, we leveraged the high resolution of single-cell technologies to redefine monocytes into eight subtypes, thereby gaining insight into the pathogenic role of monocyte subtypes in BD.

Our experiments showed the expansion of C1qhi monocytes in BD patients, as well as the enhanced phagocytosis and overproduction of proinflammatory cytokines. C1q also promoted the phagocytic capabilities of human monocytes and macrophages (68, 69) and stimulated the release of inflammatory cytokines (TNF-α and IL-6) from monocytes in RA patients (70). Consistently, trajectory inference revealed that BD monocytes preferentially differentiated into C1qhi monocytes but not monocyte-derived DCs, as suggested by previous studies showing that C1q expression increased in inflammatory monocytes (71) and inhibited DC differentiation (72). Moreover, C1q plays a central role in host defense as a pattern recognition molecule that recognizes both self and nonself ligands (73–76), indicating that proinflammatory C1qhi monocytes might help BD patients defend against pathogens that are known as critical triggers of BD development (7, 31).

It remains elusive how IFN-γ signaling mediates the immune disturbances in BD, although IFN-γ levels were significantly increased in BD patients (77–79). Our study discovered that C1qhi monocytes respond to IFN-γ activation in BD patients. As a critical transcriptional modulator of IFN-γ (51), STAT1 was also prominently activated in C1qhi monocytes, similar to the activation of STAT1 in CD14+ monocytes (80) and active macrophages (81) in other immune diseases. Additionally, monocytes treated with IFN-γ showed increased C1qhi monocytes, as suggested by the previous finding that exogenous IFN-γ induced C1q messenger RNA (mRNA) synthesis in murine macrophages (82). As Th1 cells are the main IFN-γ-producing cells among CD4+ T cells, our findings of increased IFN-γ secretion and Th1 cell differentiation support the hypothesis of activated Th1-dominated immunity in BD (79). Overall, these results suggest a model wherein overtactive Th1 cells in BD patients increase the serum concentration of IFN-γ, which induces the expansion of C1qhi monocytes. As C1qhi monocytes were also identified in other immune diseases, our findings may provide insight into their pathogenic role in not only BD but also numerous IFN-γ–involved diseases.

A critical unmet need in BD is the identification of therapeutic targets and laboratory indexes to aid in clinical assessment (3). We found that C1qhi monocytes expansion was tightly correlated with BD disease activity. Furthermore, the marker genes of C1qhi monocytes significantly predicted BD patients in multiple cohorts, and C1q protein levels were increased in BD serum. Of note, C1qhi monocytes responded to both immunosuppressive agents and tofacitinib, which our recent study reported as an effective and safe drug for BD patients (58). These data implied that C1qhi monocytes and their markers could be potential clinical parameters to assess therapeutic efficacy and that targeting this subset would bring therapeutic benefits to BD patients.

There are several limitations of our current study. First, due to the low prevalence of BD, the limited number of BD patients in the scRNA-seq data may introduce statistical bias. Hence, we verified our findings by combining both experiments and additional analyses of public cohorts. Second, we merely focused on immune cells from circulating blood, and further study of BD lesions will improve understanding of the immune landscape of BD. Third, functional phenotypes and markers of the other monocyte subtypes will also need to be confirmed in the future.

In conclusion, our study delineates the immune landscape of BD at single-cell resolution, identifies the discriminative markers and transitional dynamics of monocyte subtypes, and pinpoints the proinflammatory contribution of C1qhi monocytes in BD. Our data provide a valuable scRNA-seq resource for BD-related studies and shed light on potential targets for targeted therapies and clinical assessment of BD.

Materials and Methods

Peripheral blood samples were collected from 52 BD patients who fulfilled the 2013 International Criteria for BD (83) and 56 HCs (Dataset S1). Sixteen samples were used for scRNA-seq (four BD and four HC samples for individual scRNA-seq data), and 19 samples were used for bulk RNA-seq (9 BD and 10 HC). The study was approved by the ethical committee of the Peking Union Medical College Hospital (JS-3418). All subjects provided written informed consent.

Detailed methods are provided in SI Appendix.
