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Research Article

Abnormal glucose and lipid metabolism promotes disrupted differentiation of T and B cell subsets in Behçet's disease

Minghao Li[1,2,3,4,†], Ping Li[5,6,†], Xin Wang[5], Lijie Wang[1,2,3,4], Guanmin Gao[7], Guosheng Jiang[8], Tingting Liu[9,*], Wei Lin[1,2,3,4,*]

[1]Department of Rheumatology and Autoimmunology, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, China
[2]School of Clinical and Basic Medicine, Shandong First Medical University &Shandong Academy of Medical Sciences, Jinan, China
[3]Shandong Key Laboratory of Rheumatic Disease and Translational Medicine, Shandong Medicine and Health Key Laboratory of Rheumatism, The First Affiliated Hospital of Shandong First Medical University, Jinan, China
[4]Department of Critical-care Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China
[5]Department of Clinical Laboratory, Qilu Hospital of Shandong University, Jinan, China
[6]Shandong Engineering Research Center of Biomarker and artificial Intelligence Application, Jinan, China
[7]Department of Rheumatology and Immunology, The First Affiliated Hospital of Zhengzhou University, #1 Jianshe East Road, Zhengzhou, China
[8]Zibo First People's Hospital, Shandong, China
[9]Shandong Eye Hospital, State Key Laboratory Cultivation Base, Shandong Provincial Key Laboratory of Ophthalmology, Shandong Eye Institute, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China

[†]These authors contributed equally to this work.
[*]Corresponding authors. Department of Rheumatology and Autoimmunology, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, jingshi Road 16766#, Jinan, China. E-mail: weilin11@fudan.edu.cn

Abstract

Introduction:

Behçet's disease (BD) is a chronic, systemic inflammatory condition characterized by recurrent immune dysregulation.

Materials & Methods: This study conducted a comprehensive analysis of immune cell subsets, metabolic markers, and their interplay in BD patients. Using multiparametric flow cytometry, we identified elevated Th1 cells, senescent $CD8^+$ T cells, and abnormal B cell activation as hallmarks of the chronic inflammatory state in BD.

Results: Despite immunotherapy, innate immune activation persisted, with increased mature NK cells, γδT1 cells, and conventional dendritic cells (cDCs), alongside reduced plasmacytoid dendritic cells (pDCs). Elevated glucose (GLU) and triacylglycerol (TAG) levels in BD patients correlated with increased Th1 cells, functional $CD8^+$ T cells, and B cell activation. In vitro experiments demonstrated that GLU and TAG promote Th1 differentiation, $CD8^+$ T cell activation, and B cell antibody production, revealing their role as drivers of immune dysregulation.

Conclusion: These findings underscore the intricate relationship between metabolic dysregulation and immune dysfunction in BD, highlighting potential diagnostic and therapeutic targets. Our study provides critical insights into BD pathogenesis, offering a foundation for optimizing disease management and monitoring immune and metabolic markers for improved patient outcomes.

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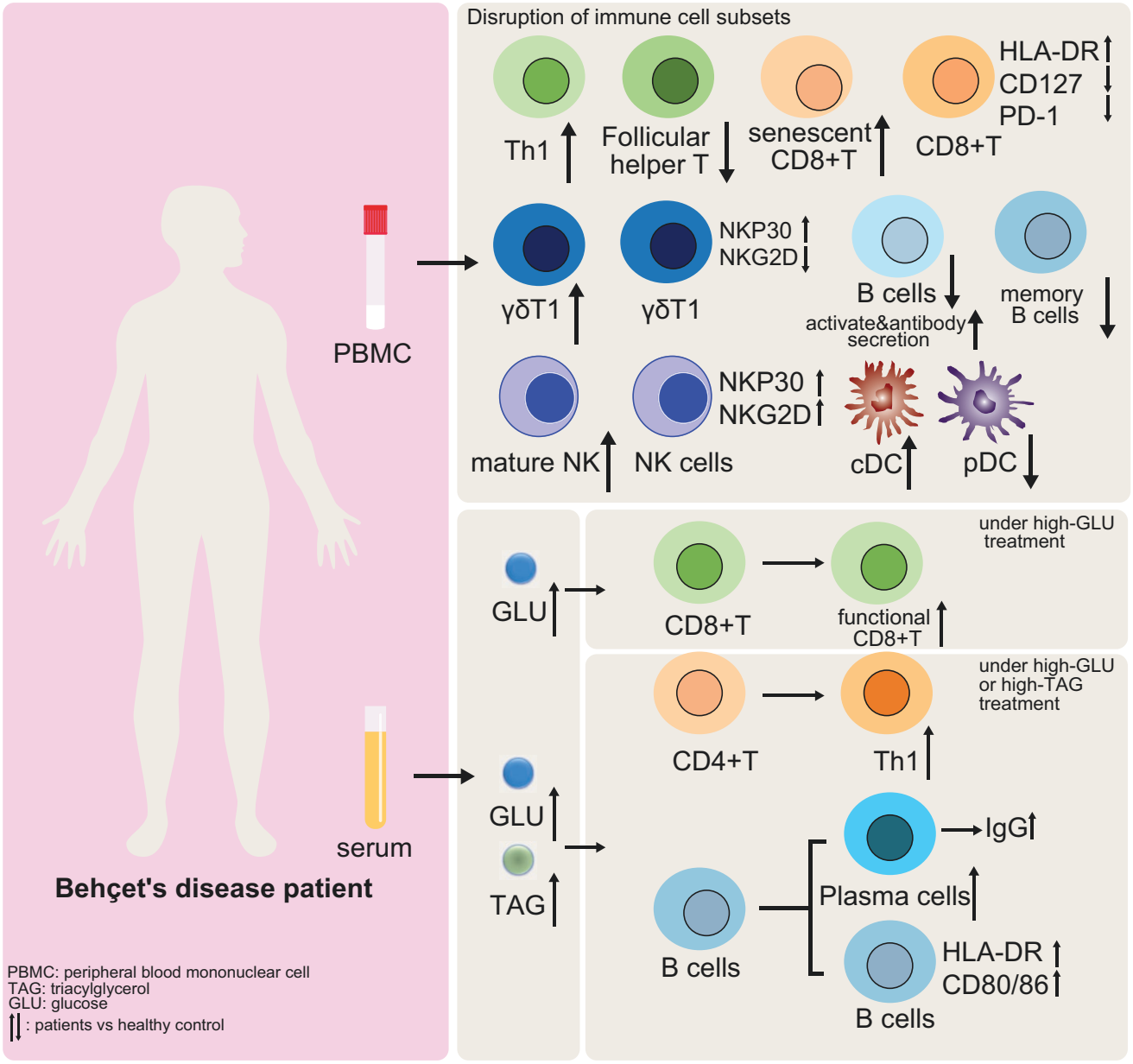
Graphical Abstract



Keywords: Behçet's disease; immune dysregulation; glucose metabolism; triglyceride metabolism; Th1; B cells

Introduction

Behçet's disease (BD) is a multisystem immune-inflammatory disorder characterized by recurrent mucocutaneous ulcers, ocular involvement, and skin lesions [1]. Its most common clinical features include recurrent orogenital ulcers and inflammatory eye lesions [2]. BD is a rare disease, with prevalence rates ranging from 20 to 420 per 100,000 in Turkey and 80 per 100,000 in Iran to 0.64 per 100,000 in the UK [3]. In East Asia, the prevalence is estimated at 13.5 to 27 per 100,000 [4]. BD lacks highly specific diagnostic methods and is generally diagnosed based on clinical criteria: patients experience ≥3 episodes of oral ulcers within a year, along with genital ulcers, uveitis, and positive skin pathergy reactions [5]. Although numerous studies have reported that immune dysregulation caused by genetic, environmental, and infectious factors leads to inflammation and clinical symptoms, the pathogenesis of BD remains unclear [6]. Consequently, the atypical symptoms and uncertain pathogenic mechanisms present challenges for clinical diagnosis and treatment, highlighting the need for precise laboratory tests to improve diagnostic and therapeutic capabilities.

Immune dysfunction is a hallmark in genetically predisposed hosts. Both innate and adaptive immunity (including B and T cells) are activated in BD [7, 8]. Dysregulation of innate immunity is marked by hyperactivation of neutrophils, monocytes, natural killer (NK) cells, and γδT cells. The activation of pathogen recognition receptors (PRRs), such as toll-like receptors (TLRs), particularly TLR2, TLR4, and TLR5, plays a crucial role in this process. These receptors, through the NF-κB signaling pathway, drive the excessive

production of pro-inflammatory cytokines [9]. NK cells in BD patients exhibit a skewed phenotype, with increased NK1 subsets during active disease and elevated NK2 subsets during remission, as well as altered cytokine production profiles correlating with disease activity [10–12]. Monocyte subsets, particularly C1q-hi monocytes induced by IFN-γ, are expanded and dysfunctional in BD, closely correlating with disease activity [13, 14]. γδT cells in the peripheral blood of BD patients are significantly increased, inducing IFN-γ and TNF-α production [15]. A reduced percentage of plasmacytoid dendritic cells (pDCs) in BD patients may contribute to pathogenicity by directing the immune response toward Th1 cells [16].

The expansion of Th1 and Th17 cells, along with their cytokines IFN-γ and IL-17, respectively, and a reduction in Treg cells are considered central to BD pathogenesis [1, 17]. $CD8^+$ T cells, as classical major histocompatibility complex (MHC) class I antigen-presenting cells, exhibit significant increases in BD patients, particularly in active diseases, where CD11a and CD11c expression is upregulated [18]. The expansion of pro-inflammatory $CD8^+$ Tem cells has been identified as a pathogenic factor, while $CD8^+$ senescent T cells are associated with chronic inflammation [19, 20]. B cells and autoantibody production also play a role in BD pathogenesis. Increased levels of activated and memory B cells indicate altered B cell function [16, 21]. Autoantigens, such as retinal S-antigen in BD uveitis, and elevated cerebrospinal fluid immunoglobulins suggest that B cells are involved in autoimmune responses [22]. The efficacy of B cell depletion therapy with anti-CD20 monoclonal antibodies in treating BD-associated uveitis further underscores their role in the disease [23].

Notably, immune dysregulation is observed in both active and resting BD patients, though the mechanisms remain unclear. Recent studies indicate that BD patients exhibit metabolic abnormalities, including reduced 25(OH) vitamin D levels and elevated lipoprotein, blood glucose, and triglyceride levels [4, 24, 25]. Elevated triglyceride levels have been shown to amplify Th17-driven inflammation in BD, implicating metabolic dysregulation as a contributor to immune dysfunction [25]. Further research is needed to clarify how these metabolic factors influence immune dysregulation.

In this study, we conducted a comprehensive analysis of multiple immune cell subsets to evaluate the immune status of BD patients. Blood biochemical indicators were measured and validated to identify factors contributing to immune dysfunction. Our findings aim to provide new insights for the diagnosis and treatment of BD.

Materials and methods

Study population

Thirty-eight BD patients (aged 34–68 years) and 42 healthy controls (HCs, aged 33–71 years) were included in this study. The patients and HCs were matched by age and sex and recruited from Shandong First Medical University Affiliated Ophthalmology Hospital, Zhengzhou First Affiliated Hospital, and Qilu Hospital of Shandong University. BD was strictly diagnosed by rheumatologists following the diagnostic criteria developed by the International Study Group for Behçet's Disease [5]. Detailed demographic and clinical information for BD patients is provided in Supplementary Table 1. The research was approved by the ethics committee of Shandong First Medical University. All participants provided informed written consent after being briefed on the study.

Sample collection and clinical laboratory tests

Fresh whole blood samples (10 ml) were collected via venipuncture into anticoagulant-free blood collection tubes. The supernatant was isolated by centrifugation at 2000 × g for 10 minutes, and 1 ml of the supernatant was used to measure C-reactive protein (CRP), glucose (GLU), triglycerides (TAG), immunoglobulin A (IgA), IgG, IgM, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total Cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Additionally, 3 ml of fresh blood was centrifuged to isolate peripheral blood mononuclear cells (PBMCs) for flow cytometry. Remaining blood cells were stored at −80°C for further analysis.

Flow cytometry (FCM)

Peripheral blood samples from all participants were collected in heparinized tubes and processed within 24 hours. Samples were diluted 1:1 with PBS buffer, overlaid on Ficoll-Hypaque media (Pharmacia), and centrifuged for PBMC isolation. Cells were washed in RPMI 1640 medium supplemented with 10% FBS (Gibco) and analyzed immediately via multiparametric flow cytometry. Experiments followed the manufacturer's instructions and were conducted by Purui Biological, Inc. A list of flow cytometry antibodies was provided in Supplementary Table 2. Flow cytometry was performed on a BD LSRFortessa X-20, and data were analyzed using FlowJo V10 software (Tree Star). Representative fluorescence gating strategies and subset analyses are shown in Supplementary Fig. S1 and Supplementary Table 3. Statistical results are provided in Supplementary Table 4.

Isolation and treatment of $CD4^+$T cells, $CD8^+$T cells, and B cells

PBMCs were isolated from fresh blood using the aforementioned protocol. $CD4^+$ T cells, $CD8^+$ T cells, and B cells were labeled with anti-human CD3, CD4, CD8, and CD19 antibodies, respectively, and purified using a BD AriaIII flow cytometer. $CD4^+$ and $CD8^+$ T cells were stimulated with anti-CD3/CD28 antibodies (eBioscience, USA), while B cells were stimulated with 100 ng/ml CD40L (MedChemExpress) and Goat $F(ab')^2$ anti-human IgM (SouthernBiotech, USA). Cells were cultured in RPMI 1640 medium containing 100 U/ml penicillin/streptomycin and 10% FBS.

For triglyceride or glucose treatments, cells were treated for five days with 2.5 mM capric triglyceride (MedChemExpress, USA) or 20 mM high glucose. For intracellular cytokine staining, 1×10^6 $CD4^+$ or $CD8^+$ T cells were stimulated with 10 ng/ml phorbol 12-myristate 13-acetate (PMA), 1 μg/ml ionomycin, and 10 μg/ml brefeldin A (all from MedChemExpress) for 6 hours. Cells were collected, washed, fixed, and permeabilized before staining with anti-human CD4, CD8-APC, IFN-γ, IL-17A, and CD28 antibodies. For B cell staining, cultured cells were labeled with anti-human CD19, IgD, IgM, CD24, CD27, and CD38 antibodies (all purchased from BioLegend, USA).

scRNA-seq acquisition and analysis

Single-cell RNA sequencing (scRNA-seq; GSE198616) data were retrieved from the GEO database. This dataset included

samples from four BD patients and four HCs, derived from peripheral blood samples of Chinese individuals. Quality control, data cleaning, and analysis were performed using the R package Seurat (v4.3.0), incorporating PCA, clustering analysis, UMAP-based dimensionality reduction, and cell type annotation for BD and HC groups. Differential gene analysis for each cell subpopulation between BD and HC groups was conducted using the FindMarkers function. Gene Ontology (GO) enrichment analyses were performed with the "clusterProfiler" package, and results were visualized using the ggplot2 (v3.4.4) package.

Statistics

Data analysis was conducted using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The difference between the two groups was compared using two-tailed Student's *t*-tests and Wilcoxon rank-sum test for non-parametric data. Correlation between Clinical parameters and the proportion of cell subsets was tested using the Pearson's parametric correlation coefficient test or Spearman's rank correlation coefficient test. Data are presented as the mean ± standard deviation (SD). Statistical significance was defined as ns $P \geq .05$, $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

Results

General lymphocyte composition analysis reveals elevated Th1 subsets and disordered CD8$^+$ T cell subpopulations in BD patients

To verify changes in immune cell subpopulations in Chinese BD patients, multicolor flow cytometry was used to analyze lymphocyte subsets from 38 BD patients and 42 healthy controls (HCs). Of these BD patients, 63.2% were undergoing immunotherapy and taking immunosuppressants at the time of blood collection. All patients had involvement of one or more organs or tissues but did not exhibit active manifestations, such as genital ulcers, skin lesions, uveitis, or arthritis requiring treatment modification at the time of blood collection (Supplementary Table 1). Although among them, 11 patients exhibited chronic active inflammation and organ damage (e.g. uveitis) at sampling collection (Supplementary Table 1), all of them were under corticosteroids or immunosuppressants treatment with atypical inflammatory symptoms. So, we believe that these BD patients were in inactivated phase.

The proportion of total B cells in the peripheral blood of BD patients was significantly decreased, while DC proportions were elevated (Fig. 1A). Although the total T cell proportions in BD patients were not different from that in HCs (Fig. 1A), their T cell subsets showed marked abnormalities. Specifically, the CD4/CD8 ratio in BD was lower than that in HCs, indicating disordered T cell subsets (Fig. 1B). The proportions of Th (CD3$^+$CD4$^+$CXCR5$^-$) and Tfh (CD3$^+$CD4$^+$CXCR5$^+$) cells among CD4$^+$ T cells were reduced, whereas Tc (CD8$^+$CCR5$^-$) cell proportions among CD8$^+$ T cells were increased compared to HCs (Fig. 1C). Further analysis showed a significant increase in Tfh2 (CD3$^+$CD4$^+$CXCR5$^+$CXCR3$^-$CCR4$^+$) cells within Tfh subsets and Tc1 (CD3$^+$CD8$^+$CXCR5$^-$CXCR3$^+$CCR4$^-$) cells within Tc subsets, as well as Th1 (CD3$^+$CD4$^+$CXCR5$^-$CXCR3$^+$CCR4$^-$) cells within Th subsets in BDs. Conversely, Tfh17 (CD3$^+$CD4$^+$CXCR5$^+$CXCR3$^-$CCR4$^-$CCR6$^+$) cells in Tfh subsets and Tc2 (CD3$^+$CD8$^+$CXCR5$^-$CXCR3$^-$CCR4$^+$) cells in Tc subsets were decreased in BD patients compared to HCs (Fig. 1D). These findings suggest persistent CD8$^+$ T cell subpopulation abnormalities and elevated Th1 levels in BD patients despite treatment.

Functional CD8$^+$ T cells (CD28$^+$CD8$^+$ T cells) were decreased, while senescent CD8$^+$ T cells (CD28$^-$CD8$^+$ T cells) were increased in BD patients compared to HCs (Fig. 1E). This increase in senescent CD8$^+$ T cells reflects chronic inflammation in BD patients. HLA-DR is a marker of mature T cell activation, particularly during the late stages of activation [26], and it was highly expressed on CD8$^+$ T cells in BD patients (Fig. 1F). Conversely, PD1$^+$CD8$^+$ T cells were downregulated in BD patients (Fig. 1G).

CD45RA and CCR7 were used to identify different maturation and memory states of CD8$^+$ T cells. Central memory T (TCM, CCR7$^+$CD45RA$^-$) and terminally differentiated effector memory T (TEMRA, CCR7$^-$CD45RA$^+$) subsets were elevated in BD patients, while effector memory T (TEM, CD3$^+$CD8$^+$CCR7$^-$CD45RA$^-$) subsets were reduced (Fig. 1H). CD127 expression, indicative of immune cell response capacity to viral resistance [27], was reduced in CD127$^+$CD8$^+$ TEMRA subsets in BD patients compared to HCs (Fig. 1I), suggesting impaired antiviral capabilities in BD patients.

Overall, these findings indicate that Th1 and CD8$^+$ T cell subsets, including Tc1 and CD8$^+$ TEMRA, are highly activated in BD patients. These subsets are likely key pathogenic factors contributing to recurrent attacks. Additionally, the increased senescent CD8$^+$ T cell population reflects the chronic inflammatory nature of BD.

Humoral immune impairment and aberrant innate immunity in BD patients

The proportions of total B cells (CD3$^-$CD19$^+$) and memory B cells (CD3$^-$CD19$^+$CD24$^+$CD27$^+$CD38$^+$IgD$^+$IgM$^+$) were significantly reduced in BD patients (Figs. 1A and 2A). In contrast, transformed B cells (CD19$^+$IgM$^+$ IgD$^-$CD27$^-$CD38$^+$CD24$^+$), B10 cells (CD19$^+$IgM$^+$IgD$^+$CD24$^+$CD27$^+$), and immature regulatory B cells (CD3$^-$CD19$^+$IgD$^+$IgM$^+$CD27$^-$CD38$^+$CD24$^+$) were significantly elevated in BD patients compared to HCs (Fig. 2B–D). The proportion of plasma B cells remained unchanged (Fig. 2E), but total serum antibody levels (IgG, IgA, IgM) were higher in BD patients than in HCs (Fig. 2F). These findings indicate that, despite controlling the proportion of B cells following treatment, antibody production levels suggest persistent overactivation of B cell function.

NK cells have been reported to be associated with BD pathogenesis [28, 29]. Although the percentage of NK cells (CD3$^-$CD56$^+$) showed no significant change, the proportion of immature NK cells (CD3$^-$CD56^{bright}) decreased, and mature NK cells (CD3$^-$CD56^{dim}) were increased in BD patients compared to HCs (Figs. 1A and 3A). Markers of NK cell activation, including NKG2D, NKP30, and NKP46, were upregulated in BD patients (Fig. 3B). Meanwhile, the proportion of functionally blocked NK cells (CD3$^-$CD56$^+$NKB1$^+$CD29$^-$) was downregulated in BD patients (Fig. 3C). These findings suggest that the cytotoxic CD56^{dim} NK subset is functionally activated, while the reduced presence of regulatory NK cells exacerbates the pathogenic role of cytotoxic NK cells in BD. Consistent with previous studies [30], a decreased frequency of NKT cells was observed in PBMCs from BD patients compared to HCs (Fig. 3D).

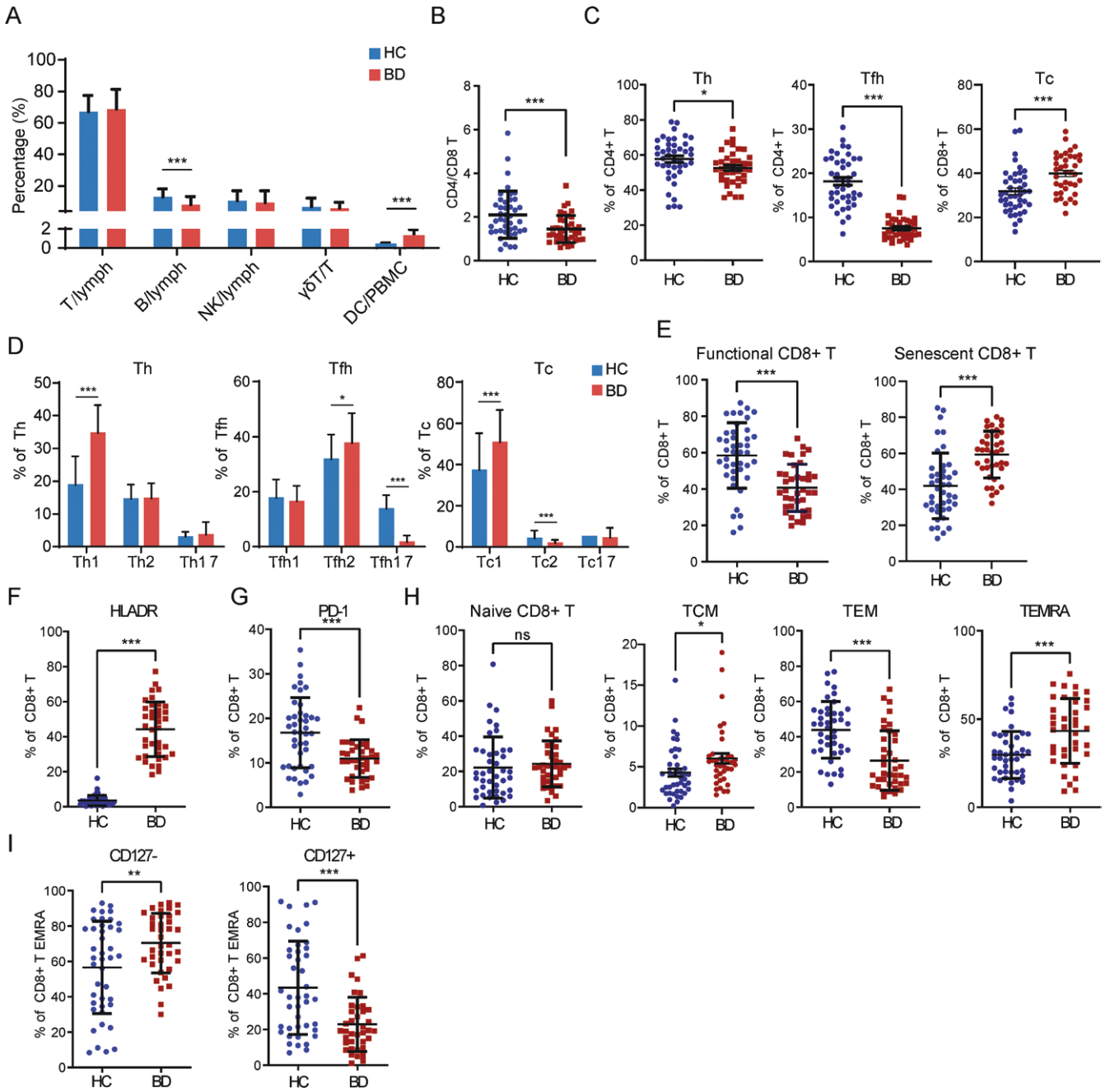


Figure 1. The distribution of lymphocyte subtypes in healthy controls and Behçet's disease patients. (**A**) Percentage of T cells, B cells, and NK cells in the lymphocytes of healthy controls (HC) and Behçet's disease patients (BD). Percentage of γδT cells in T cells of HCs and BDs. Percentage of DCs in PBMCs of HCs and BDs. (**B**) CD4/CD8 ratio in HCs and BDs. (**C**) Percentage of Th and Tfh in $CD4^+$T cells and percentage of Tc in $CD8^+$ T cells of HCs and BDs. (**D**) Percentage of Th1/Th2/Th17 in Th cells, Tfh1/Tfh2/Tfh17 in Tfh cells, and Tc1/Tc2/ Tc17 in Tc cells of HCs and BDs. (**E**) Percentage of functional and senescent $CD8^+$ T in $CD8^+$T cells of HCs and BDs. The HLADR (**F**) and PD-1 (**G**) expression on $CD8^+$ T cells of HCs and BDs. (**H**) Proportion of naïve $CD8^+$ T cells, Tcm, Tem, and Temra in $CD8^+$ T cells of HCs and BDs. **(I)** The CD127 expression on $CD8^+$ Temra of HCs and BDs. Experiments were performed on 42 healthy subjects and 38 patients, and shown as mean ± SD, data were analyzed by two tails *t*-test. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$, ns means no difference.

γδT cells, another critical subset in BD, possess unconventional TCRs that recognize peptides, lipids, or other molecules and mediate antiviral and antitumor effects without MHC restriction [31]. Our results showed no difference in the proportion of γδT cells ($CD3^+\gamma\delta^+$) between BD patients and HCs (Fig. 1A). However, the γδT1 subset ($CD3^+\gamma\delta^+VD1^+VD2^-$), typically resident in the thymus and mucosal tissues, was upregulated in BD patients (Figs. 1A and 3E). This suggests that adaptive γδT1 cells in the peripheral blood may rapidly proliferate in response to antigens during disease progression [32]. Furthermore, NKP30 expression was upregulated in γδT1 and γδT2 cells, while NKG2D expression was downregulated in both subsets in BD patients (Fig. 3F and G). These findings indicate activation of γδT cell subsets in BD patients despite unchanged γδT2 cell proportions.

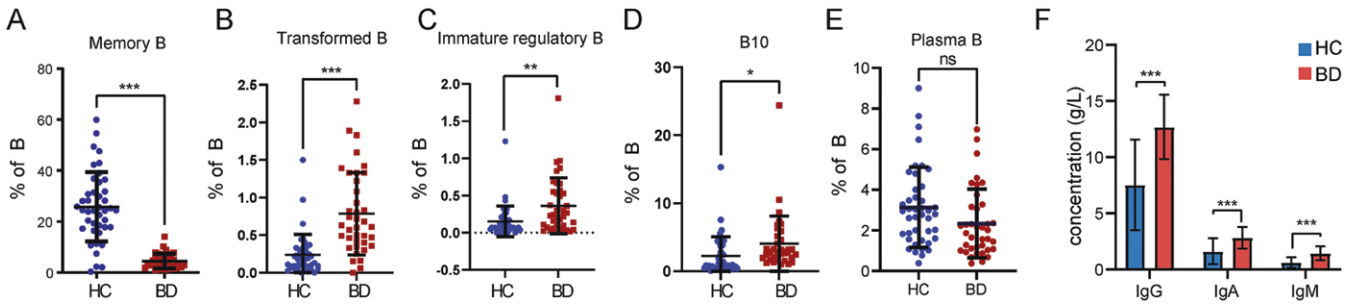


Figure 2. The distribution of B cell subtypes in healthy controls and Behçet's disease patients. (**A-E**) Comparison of the percentage of memory B cells (**A**), transformed B cells (**B**), immature regulatory B cells (**C**), B10 cells (**D**) and plasma B cells (**E**) in B cells of HCs and BDs. (**F**) The concentration of IgG, IgA, and IgM in serum of HCs and BDs. Experiments were performed on 42 healthy subjects and 38 patients, and shown as mean ± SD, data were analyzed by two tails *t*-test. **P* < .05, ***P* < .01, ****P* < .001, ns means no significant.

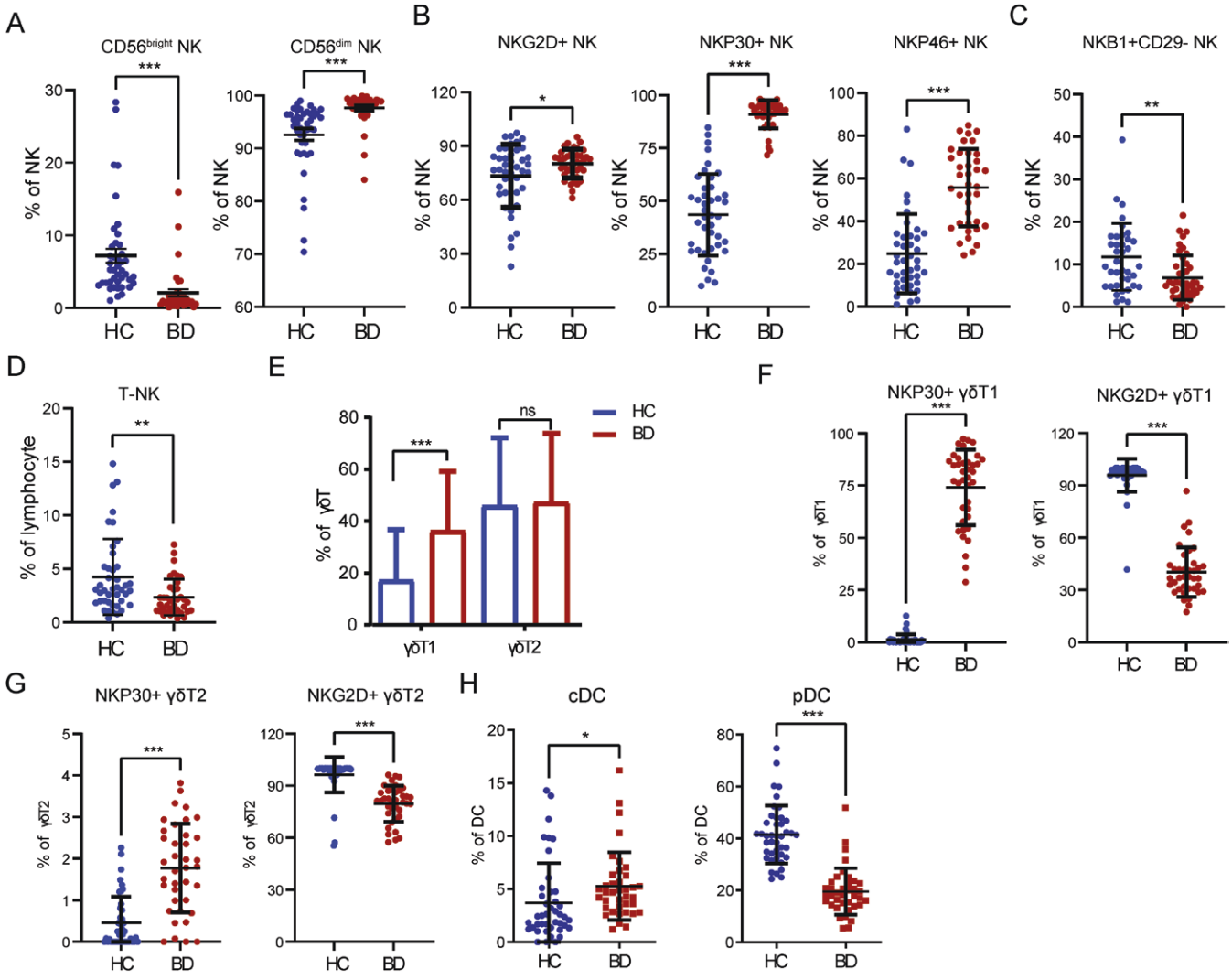


Figure 3. The distribution of NK cell, γδT and DC cell subtypes in healthy controls and Behçet's disease patients. (**A**) Percentage of CD56^{dim} NK cells in NK cells of HCs and BDs. (**B**) The NKG2D, NKP30, and NKP46 expression in NK cells of HCs and BDs. (**C**) Percentage of NKB1^{+}CD29^{-} NK cells in NK cells of HCs and BDs. (**D**) Percentage of T-NK in lymphocytes of HCs and BDs. (**E**) Percentage of γδT1 and γδT2 cells in γδT cells of HCs and BDs. **(F)** Percentage of NKG2D^{+} or NKP30^{+} γδT1 cells in γδT1 of HCs and BDs. (**G**) Percentage of NKG2D^{+} or NKP30^{+} γδT2 cells in γδT2 of HCs and BDs. (**H**) Percentage of cDCs and pDCs in total DCs of HCs and BDs. Experiments were performed on 42 healthy subjects and 38 patients, and shown as mean ± SD, data were analyzed by two tails *t*-test. **P* < .05, ***P* < .01, ****P* < .001.

DCs, as potent antigen-presenting cells, play a significant role in BD progression [33]. Our flow cytometry analysis revealed an increased proportion of DCs in the peripheral blood of BD patients, with an upregulated cDC subgroup and a downregulated pDC subgroup (Fig. 3H). Such abnormal proportions of DC subgroups may contribute to inducing specific T cell responses in BD patients.

In summary, aberrant innate immune responses, including NK cell, γδT cell, and DC abnormalities, were associated with BD pathogenesis.

BD patients exhibit dysregulated lipid metabolism correlating with altered immune cell subsets

All BD patients exhibited one or more abnormal indicators including elevated LDL-C concentration, CHO, AST, TAG, and GLU or decreased HDL-C, suggesting abnormal liver function and lipid metabolism disorders in BD (Fig. 4A and Supplementary Table 5). Among these indicators, AST levels were higher in BD patients than in HCs, with 43.6% of BD patients exceeding the normal reference range. TAG levels were also elevated in BD patients compared to HCs, with 64.1% of BD patients showing high triglyceride levels. Additionally, GLU levels were elevated in BD patients, with 23.1% presenting hyperglycemia (Fig. 4A), suggesting that BD patients experience metabolic abnormalities.

To examine whether elevated TAG or GLU levels were associated with altered immune cell subsets, we analyzed the correlation between these metabolites and immune cell proportions in BD patients. The proportion of functional $CD8^+$ T cells in BD patients positively correlated with TAG and GLU concentrations, whereas senescent $CD8^+$ T cells negatively correlated with these metabolites (Fig. 4B). Additionally, the proportion of Th1 cells was positively correlated with GLU levels in BD patients (Fig. 4C), with an increased percentage observed in BD patients compared to HCs (Fig. 1D). GLU levels were also positively correlated with the proportion of B cells in BD patients (Fig. 4D). These findings suggest that dysregulated glucose and lipid metabolism correlate with alterations in T and B cell subsets in BD.

TAG and GLU promoteT cell subset differentiation

To evaluate the effects of glucose and TAG on T and B cells, we isolated T and B cells from the peripheral blood of three healthy individuals and stimulated them with TAG and GLU in vitro. High GLU levels, but not other factors, increased the proportion of functional $CD8^+$ T cells ($CD28^+CD8^+$ T cells) among $CD8^+$ T cells (Fig. 5A). Although TAG did not affect $CD28^+CD8^+$ T cell production, both TAG and high GLU stimulated granzyme B (GZMB) production in $CD8^+$ T cells, suggesting that TAG and GLU promote inflammatory $CD8^+$ T cells. High GLU levels promoted Th1 cell production but not Th17 cells (Fig. 5B), while TAG treatment also enhanced Th1 cell production. Moreover, under high GLU or TAG conditions, $CD4^+$T and $CD8^+$T from BD patients also showed the same properties (Supplementary Fig. S2). These results indicate that elevated GLU and TAG levels in BD patients may be key risk factors contributing to Th1 cell differentiation and CD28 expression on $CD8^+$ T cells.

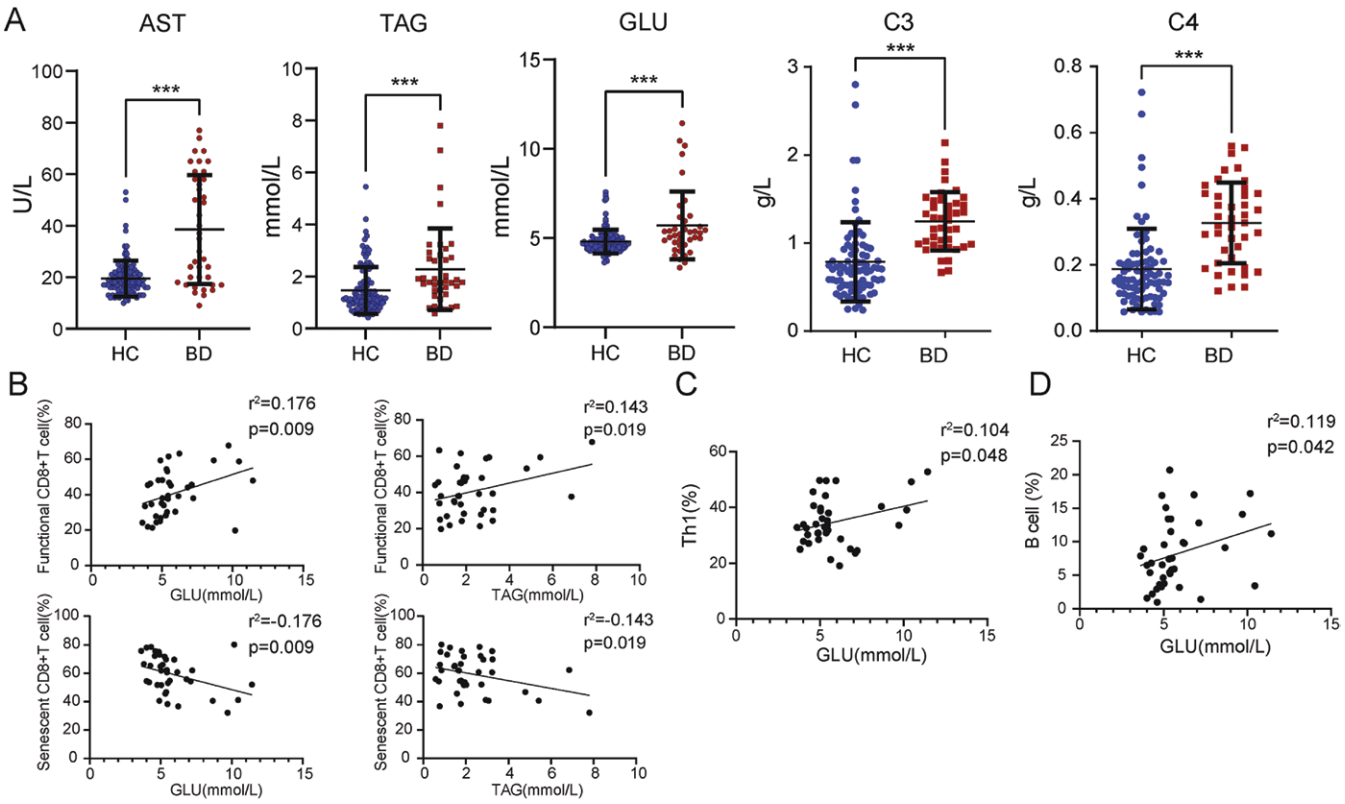


Figure 4. Dysregulation of lipid metabolism is associated with Behcet's disease. (**A**) Serum concentrations of AST, TAG, GLU, C3, and C4 in HCs and BDs. Experiments were performed on 42 healthy subjects and 38 patients. Data was analyzed by two tails *t*-test. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$. (**B**) Pearson correlation analysis between the percentage of functional $CD8^+$ T cells or senescent $CD8^+$ T cells and serum GLU or TAG concentrations in BD patients. (**C-D**) Pearson correlations analysis between the percentage of Th1 cell or B cell and serum GLU concentrations in BD patients. Experiments were performed on 38 patients, and shown as mean ± SD, data were analyzed by Pearson's parametric correlation coefficient test.

TAG and GLU promote B cell activation

Although the total number of B cells in both acute and resting BD patients did not significantly change [16, 34], antibody production was increased, possibly due to excessive B cell activation. Analysis of single-cell RNA sequencing data from PBMCs of BD patients revealed enrichment of pathways related to "activation of immune response," "immune response activating signal transduction," and "positive regulation of lymphocyte activation" in B cell subsets (Fig. 6A). Additionally, genes associated with inflammation and activation, such as *HLA-DQA*, *CXCR4* and *CD83*, were upregulated in B cells from BD patients compared to HCs

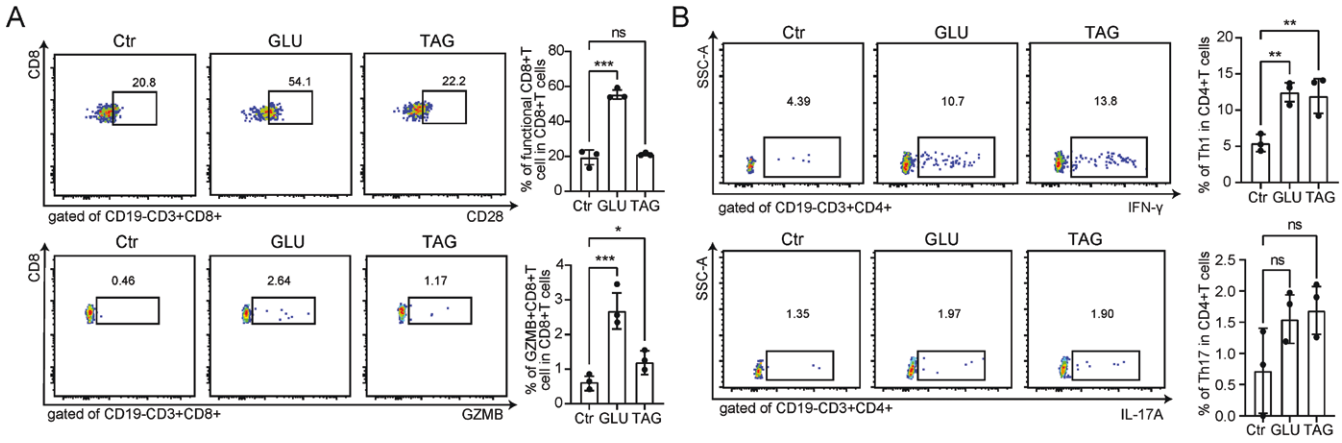


Figure 5. TAG and GLU promote T cell subset differentiation. **(A)** Percentage of $CD28^+$ $CD8^+$ T cells and $GZMB^+CD8^+$ T cells in TAG-treated, GLU-treated, or untreated control $CD8^+$ T cells (Ctr). **(B)** Percentage of Th1 and Th17 cells in TAG-treated, GLU-treated, or untreated control $CD8^+$ T cells (Ctr). Experiments were performed by using PBMC from 3 healthy donors, and shown as mean ± SD, data were analyzed by two tails *t*-test. $^{**}P < .01$, $^{***}P < .001$, ns means no significance.

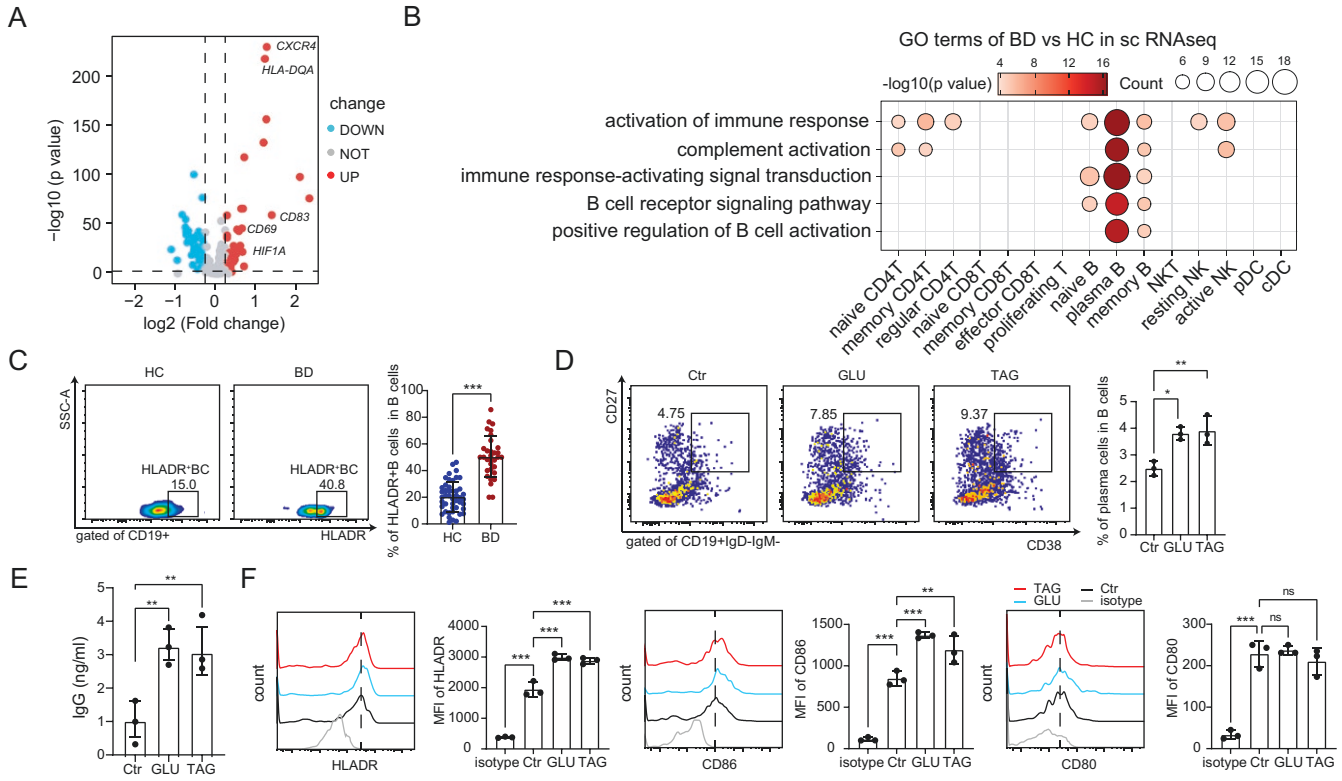


Figure 6. TAG and GLU promote B cell activation. (**A**) Volcano plot showing the differentially expressed genes in B cells from BD group compared to HC group. (**B**) Bubble plot showing GO-enriched terms from BD compared to HC group. (A-B) Experiments were performed by PBMC from 3 healthy subjects and 3 BD patients. (**C**) The percentage of $HLADR^+$ B cells within B cells from HCs and BDs. Experiments were performed by PBMC from 42 healthy subjects and 38 BD patients, and shown as mean ± SD, data were analyzed by two tails *t*-test. $^{***}P < .001$. (**D**) Comparison of plasma cells frequencies in TAG-treated, GLU-treated, or untreated control B cells. (**E**) the concentration of IgG in cell culture supernatant of TAG-treated, GLU-treated, or untreated control B cells. (**F**) MFI of HLADR, CD80, CD86 in TAG-treated, GLU-treated, or untreated control B cells. (D-F) Experiments were performed by PBMC from 3 healthy donors, and shown as mean ± SD, data were analyzed by two tails t-test. $^{**}P<0.01$, $^{***}P<0.001$, ns means no significance.

(Fig. 6B). Flow cytometry analysis showed that the proportion of HLADR$^+$ B cells was higher in BD patients than in HC (Fig. 6C). In vitro experiments, TAG or GLU treatment further promoted differentiation of plasma cells (Fig. 6D), and higher levels of IgG antibodies production in the supernatant of B cells from health control (Fig. 6E). Additionally, under high glucose or high triglyceride conditions, B cells showed increased activation markers HLA-DR, CD80, and CD86 in B cells from health control (Fig. 6F). Moreover, the same results were found in B cells from BD patients (Supplementary Fig. S2C–E). These findings suggest that elevated TAG and GLU levels may act as potential drivers of B cell activation in BD patients.

Discussion

Behçet's disease is a chronic, systemic, and recurrent inflammatory condition characterized by hyperactive Th1 and Th17 immune responses. Using multiparametric flow cytometry, we conducted a comprehensive analysis of immune cell subsets in BD patients, revealing that elevated Th1 subsets and senescent CD8$^+$ T cells signify a chronic inflammatory state. Additionally, intrinsic immune activation was evident, with increased mature NK cells, elevated γδT1 cells, and decreased pDCs. Although the percentage of total B cell was reduced, transformed B cells and antibody levels (IgG, IgA, IgM) were elevated. Increased GLU and TAG levels in these patients were identified as significant factors contributing to the increase in Th1 ratios, B cells activation, and IgG levels.

Our findings show that while the overall proportions of total T and B cells in the peripheral blood of BD patients did not differ significantly from those of HCs, cell subsets such as Th1, Tfh2, Tc1, senescent CD8$^+$ T cells, CD8$^+$ TCM cells, and CD8$^+$ TEMRA cells were elevated, indicating chronic inflammation in BD patients. The role of Tfh2 and TEMRA in the pathogenicity of BD has not been reported, which need further studying. Furthermore, our data demonstrated increased HLA-DR expression on CD8$^+$ T cells, alongside decreased PD-1 and CD127 expression, suggesting disruption within CD8$^+$ T cell subsets. The functional CD8$^+$ T cell subset (CD28$^+$CD8$^+$ T cells) was reduced, while senescent CD8$^+$ T cells (CD28$^-$CD8$^+$ T cells) were elevated in BD, which is consistent with other autoimmune diseases [20, 35, 36] and suggests the presence of sustained immune activation and inflammatory states in BD patients. Reduced CD28 expression has been recognized as a marker of T cell exhaustion. Despite this, CD28$^-$CD8$^+$ T cells have been shown to retain cytotoxic functions, including perforin and granzyme production, with high cytotoxic capacity [37–40]. The loss of CD28 reduces the specificity of T cell responses to environmental stimuli, linking CD28 deficiency to T cell dysfunction and dysregulation. Thus, CD28$^-$CD8$^+$ T cells may not only represent traditional senescent lymphocytes but could also contribute to the inflammatory and immune responses observed in autoimmune diseases, such as BD, potentially correlating with more severe manifestations. Further studies are needed to explore the role of CD28$^-$CD8$^+$ T cells in BD patients.

While abnormal B cell distribution in the peripheral blood of BD patients has been reported [21], the distribution of specific B cell subsets has not been fully characterized in BD. Our findings provide novel insights into subset-specific alterations: although total antibody levels (IgG, IgA, IgM) in BD patients were significantly elevated, the overall proportion of B cells and memory B cells decreased. This observation aligns with previous studies [14, 41], suggesting potential suppression of B cell function, potentially associated with reduced Tfh cells in BD patients. However, single-cell RNA sequencing revealed activation of memory B cells and plasma B cells. Transcriptomic analyses indicated that, despite a reduced quantity, memory B cells remained in an inflammatory state, suggesting retained functional activity. Existing studies have proposed that B cell abnormalities in BD may be linked to the disease's chronic inflammatory state, potentially affecting the generation and maintenance of memory B cells. Additionally, the reduction in memory B cells may be related to their migration to local inflammatory sites [41]. The decrease in B cell numbers, coupled with an increase in transitional and other functionally active B cells, reflects a highly stressed humoral immune system in BD patients. Although increased regulatory B cells may suppress memory B cells or other immune cells, polymorphisms in IL-10 and IL-35 associated with regulatory B cells in BD patients have been reported [42]. Thus, even with elevated regulatory B cells, effective suppression of overactivated immune responses may not be achieved. In conclusion, potential immune modulation targeting B cells could play a critical role in managing BD, addressing both the humoral immune dysregulation and the broader inflammatory responses in this disease.

In our study, despite treatment with immunosuppressive drugs, the innate immune system of BD patients remained activated. The proportion of DCs, particularly cDCs, increased, whereas pDCs decreased. Additionally, the proportion of mature NK cells was elevated and displayed an activated state. This persistent chronic inflammation in BD patients highlights a gap in previous reports [6], which failed to explain the sustained activation of innate immunity in BD. Mature NK cells (CD56^{dim}, accounting for 90% of peripheral blood NK cells) possess a high capacity for degranulation and produce perforin and granzyme B. Their function is regulated by surface activating receptors (e.g. NKG2D, NKp46, NKp30) and inhibitory receptors (e.g. Siglec-7/9). We observed that NK cells in BD patients exhibited functional activation, as indicated by the upregulation of activating receptors NKG2D and NKp30. This abnormal activation of NK cells may further exacerbate immune dysregulation in BD patients, and current immunotherapies appear ineffective in modulating this dysfunction,which need further studying to resolve.

Our study identified significant elevations in TAG and GLU levels in BD patients. These metabolic markers positively correlated with the frequency of functional CD8$^+$ T cells, underscoring the crosstalk between metabolic dysregulation and immune dysfunction in BD pathogenesis [4]. Notably, increased TAG and GLU levels promoted the expansion of functional CD8$^+$ T cells and the differentiation of Th1 cells. This indicates a complex and multidimensional relationship between metabolic status and immune function in BD. However, the overall proportion of functional CD8$^+$ T cells was decreased in BD patients, possibly due to the inhibitory effects of immunotherapy or other factors counteracting the influence of TAG or GLU on CD8$^+$ T cells. Moreover, GLU and TAG enhanced the differentiation capacity and antigen-presenting ability of B cells. Nutrients and metabolism-related molecules are crucial for B cells, which are metabolically active cells [43, 44]. Glucose has been reported to drive B cell

development and antibody production through glycolysis and oxidative phosphorylation [45, 46]. While lipid metabolism is essential for B cell development and antibody production [43], the precise mechanisms by which TAG affects antibody production remain unclear and warrant further research. Whether high glucose in patients with BD is a key factor in their immune disorder remains to be further studied.

The BD patients included in our study are in the inactive phase. Compared with previous reports about the immune status of activated BD [8, 29, 47, 48], the percentage of total T cells, NK, Th17, and gamma-delta T cells in the lymphocytes of peripheral blood returned to the same level as the normal healthy group. But, the percentage of Th1, $CD56^{dim}$ NK, DC, senescent $CD8^+$ T cells in the lymphocytes, and antibody level of BD patients were still higher than healthy control. It suggests that the current treatment strategies has a certain therapeutic effect on the disordered immune state, but due to limitations in understanding the complex mechanisms underlying acute onset [9] (e.g. neutrophils could participate in the BD patients), the imbalance of innate immunity (e.g. activated NK/γδT cells and DC subset imbalance) could not be improved even post-treatment, which may also influence activated $CD8^+$ T cells. In our cohort, 63.2% BD patients were treated by immunosuppressants, on the day of sample collection. The frequencies of decreased Th1 cells, activated Tfh, Tfh1, immature regulatory B cells, and $NKp46^+$NK cells decreased and Th2 subsets, Tfh2, and Tc2 increased in treated BD patients compared to untreated BD patients (Supplementary Fig. S3A). It suggested that the current therapy is effective in maintaining immune homeostasis. But, current immunosuppressive drugs could not alleviate the disorder of $CD56^{dim}$ NK cell, which produced more perforin and granzyme B [29]. Treating on the disordered of NK cell subsets might be prospective therapy for controlling the immune disorder of BD patients. Above all, the pathogenesis of Behcet's disease requires further research, and strategies for precise treatment and regulation of patients' immune status require further investigation. Due to the limited sample size, the immune landscape heterogeneity among different BD groups could not be thoroughly examined. Future studies with expanded sample sizes should investigate the changes in heterogeneous immune cell subsets in BD patients.

In conclusion, this is the first study to provide a comprehensive cross-sectional analysis of the immune landscape in BD patients. Immunosenescence and exhaustion of $CD8^+$ T cells, overactivation of B cells, and enhanced cytotoxicity of NK and γδT cells contribute to immune dysfunction during BD progression. Furthermore, elevated glucose and lipid levels in BD patients partially drive the overactivation of adaptive immunity. These findings offer valuable insights into identifying diagnostic markers, monitoring disease relapse, and optimizing disease management in BD.

Supplementary material

Supplementary data are available at Immunotherapy Advances online.

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Author contributions

Minghao Li (Data curation, Formal analysis, Validation, Visualization, Writing - original draft), Ping Li (Formal analysis, Investigation, Resources), Xin Wang (Formal analysis, Investigation, Resources), Lijie Wang (Methodology, Validation), Guanmin Gao (Formal analysis, Investigation, Resources), Guosheng Jiang (Investigation, Methodology, Resources), Tingting Liu (Project administration, Supervision, Writing - review & editing), and Wei Lin (Project administration, Supervision, Writing - original draft, Writing - review & editing)

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

The research was approved by the ethics committee of Shandong First Medical University. All participants provided informed written consent after being briefed on the study.

Data availability

The values for all data points shown in the figure are available upon request.

References

1. Greco A, De Virgilio A, Ralli M et al. Behçet's disease: new insights into pathophysiology, clinical features and treatment options. *Autoimmun Rev* 2018; 17:567–75. https://doi.org/10.1016/j.autrev.2017.12.006
2. Wang Q, Ma J, Gong Y et al. Sex-specific circulating unconventional neutrophils determine immunological outcome of autoinflammatory Behcet's uveitis. *Cell Discov* 2024; 10:47. https://doi.org/10.1038/s41421-024-00671-2
3. Davatchi F, Chams-Davatchi C, Shams H et al. Behcet's disease: epidemiology, clinical manifestations, and diagnosis. *Expert Rev Clin Immunol* 2017; 13:57–65. https://doi.org/10.1080/1744666X.2016.1205486
4. Chen T, Shao X, Li H et al. Association of Behcet's disease with the risk of metabolic syndrome and its components: a systematic review and meta-analysis. *Clin Exp Med* 2023; 23:2855–66. https://doi.org/10.1007/s10238-023-01044-x
5. International Study Group For Behçet's Disease. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. *Lancet* 1990; 335:1078–80.
6. van der Houwen TB, van Hagen PM, van Laar JAM. Immunopathogenesis of Behçet's disease and treatment modalities. *Semin Arthritis Rheum* 2022; 52:151956. https://doi.org/10.1016/j.semarthrit.2022.151956

7. Gül A. Behçet's disease as an autoinflammatory disorder. *Curr Drug Targets Inflamm Allergy* 2005; 4:81–3. https://doi.org/10.2174/1568010053622894
8. Hu D, Guan JL. The roles of immune cells in Behçet's disease. *Adv Rheumatol* 2023; 63:49. https://doi.org/10.1186/s42358-023-00328-w
9. van der Houwen TB, Dik WA, Goeijenbier M et al. Leukocyte toll-like receptor expression in pathergy positive and negative Behçet's disease patients. *Rheumatology (Oxford)* 2020; 59:3971–9. https://doi.org/10.1093/rheumatology/keaa251
10. Sallakci N, Tahrali I, Kucuksezer UC et al. Effect of different cytokines in combination with IL-15 on the expression of activating receptors in NK cells of patients with Behçet's disease. *Immunol Res* 2022; 70:654–66. https://doi.org/10.1007/s12026-022-09298-5
11. Aktas E, Erten G, Kucuksezer UC et al. Natural killer cells: versatile roles in autoimmune and infectious diseases. *Expert Rev Clin Immunol* 2009; 5:405–20. https://doi.org/10.1586/eci.09.27
12. Cosan F, Aktas Cetin E, Akdeniz N et al. Natural killer cell subsets and their functional activity in Behçet's disease. *Immunol Invest* 2017; 46:419–32. https://doi.org/10.1080/08820139.2017.1288240
13. Li C, Liu J, Yu X et al. Aberrant monocyte subsets in patients with Behçet's disease. *Clin Immunol* 2021; 225:108683. https://doi.org/10.1016/j.clim.2021.108683
14. Zheng W, Wang X, Liu J et al. Single-cell analyses highlight the proinflammatory contribution of C1q-high monocytes to Behçet's disease. *Proc Natl Acad Sci USA* 2022; 119:e2204289119. https://doi.org/10.1073/pnas.2204289119
15. Freysdottir J, Lau S, Fortune F. Gammadelta T cells in Behçet's disease (BD) and recurrent aphthous stomatitis (RAS). *Clin Exp Immunol* 1999; 118:451–7. https://doi.org/10.1046/j.1365-2249.1999.01069.x
16. Ekşioglu-Demiralp E, Kibaroglu A, Direskeneli H et al. Phenotypic characteristics of B cells in Behçet's disease: increased activity in B cell subsets. *J Rheumatol* 1999; 26:826–32.
17. Singh RP, Hasan S, Sharma S et al. Th17 cells in inflammation and autoimmunity. *Autoimmun Rev* 2014; 13:1174–81. https://doi.org/10.1016/j.autrev.2014.08.019
18. Park YJ, Park MJ, Park S et al. CD11c is upregulated in CD8+ T cells of patients with Behçet's disease. *Clin Exp Rheumatol* 2016; 34:S86–91.
19. Islam SMS, Kim HA, Choi B, et al. Differences in expression of human leukocyte antigen class ii subtypes and t cell subsets in Behcet's disease with arthritis. *Int J Mol Sci* 2019; 20:5044. https://doi.org/10.3390/ijms20205044
20. Yang JY, Park MJ, Park S et al. Increased senescent CD8+ T cells in the peripheral blood mononuclear cells of Behçet's disease patients. *Arch Dermatol Res* 2018; 310:127–38. https://doi.org/10.1007/s00403-017-1802-8
21. Suzuki N, Sakane T, Ueda Y et al. Abnormal B cell function in patients with Behcet's disease. *Arthritis Rheum* 1986; 29:212–9. https://doi.org/10.1002/art.1780290209
22. Takeuchi M, Usui Y, Okunuki Y et al. Immune responses to interphotoreceptor retinoid-binding protein and S-antigen in Behcet's patients with uveitis. *Invest Ophthalmol Vis Sci* 2010; 51:3067–75. https://doi.org/10.1167/iovs.09-4313
23. Evereklioglu C. Current concepts in the etiology and treatment of Behçet disease. *Surv Ophthalmol* 2005; 50:297–350. https://doi.org/10.1016/j.survophthal.2005.04.009
24. Do JE, Kwon SY, Park S et al. Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behcet's disease. *Rheumatology (Oxford)* 2008; 47:840–8. https://doi.org/10.1093/rheumatology/ken109
25. Tan S, Feng X, Liu Z et al. The pro-inflammatory effect of triglyceride on human CD4(+) T cells and experimental autoimmune uveitis. *Clin Immunol* 2022; 240:109056. https://doi.org/10.1016/j.clim.2022.109056
26. Lins L, Farias E, Brites-Alves C et al. Increased expression of CD38 and HLADR in HIV-infected patients with oral lesion. *J Med Virol* 2017; 89:1782–7. https://doi.org/10.1002/jmv.24852
27. Cellerai C, Perreau M, Rozot V et al. Proliferation capacity and cytotoxic activity are mediated by functionally and phenotypically distinct virus-specific CD8 T cells defined by interleukin-7R{alpha} (CD127) and perforin expression. *J Virol* 2010; 84:3868–78. https://doi.org/10.1128/JVI.02565-09
28. Müller C, Schernthaner G, Kovarik J et al. Natural killer cell activity and antibody-dependent cellular cytotoxicity in patients under various immunosuppressive regimens. *Clin Immunol Immunopathol* 1987; 44:12–9. https://doi.org/10.1016/0090-1229(87)90047-x
29. Hasan MS, Ryan PL, Bergmeier LA et al. Circulating NK cells and their subsets in Behçet's disease. *Clin Exp Immunol* 2017; 188:311–22. https://doi.org/10.1111/cei.12939
30. Hamzaoui K, Kamoun M, Houman H et al. Discrepancies of NKT cells expression in peripheral blood and in cerebrospinal fluid from Behçet's disease. *J Neuroimmunol* 2006; 175:160–8. https://doi.org/10.1016/j.jneuroim.2006.02.011
31. Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat Rev Immunol* 2013; 13:88–100. https://doi.org/10.1038/nri3384
32. Hu Y, Hu Q, Li Y et al. γδ T cells: origin and fate, subsets, diseases and immunotherapy. *Signal Transduct Target Ther* 2023; 8:434. https://doi.org/10.1038/s41392-023-01653-8
33. Pay S, Simsek I, Erdem H et al. Dendritic cell subsets and type I interferon system in Behçet's disease: does functional abnormality in plasmacytoid dendritic cells contribute to Th1 polarization? *Clin Exp Rheumatol* 2007; 25:S34–40.
34. Hetta HF, Mohamed AAA, Zahran AM et al. Possible role of regulatory B cells in different Behcet's disease phenotypes and therapies: first report from Egypt. *J Inflamm Res* 2021; 14:737–44. https://doi.org/10.2147/JIR.S279912
35. He J, Li Y, Chen J et al. The relationships of CD8+ T cell subsets in RA patients with disease activity and clinical parameters. *Int Immunopharmacol* 2023; 114:109399. https://doi.org/10.1016/j.intimp.2022.109399
36. Huang Y, Zheng H, Zhu Y et al. Loss of CD28 expression associates with severe T-cell exhaustion in acute myeloid leukemia. *Front Immunol* 2023; 14:1139517. https://doi.org/10.3389/fimmu.2023.1139517
37. Fiorentino S, Dalod M, Olive D et al. Predominant involvement of CD8+CD28- lymphocytes in human immunodeficiency virus-specific cytotoxic activity. *J Virol* 1996; 70:2022–6. https://doi.org/10.1128/JVI.70.3.2022-2026.1996
38. Casado JG, Soto R, DelaRosa O et al. CD8 T cells expressing NK associated receptors are increased in melanoma patients and display an effector phenotype. *Cancer Immunol Immunother* 2005; 54:1162–71. https://doi.org/10.1007/s00262-005-0682-5
39. Sun Z, Zhong W, Lu X et al. Association of Graves' disease and prevalence of circulating IFN-gamma-producing CD28(-) T cells. *J Clin Immunol* 2008; 28:464–72. https://doi.org/10.1007/s10875-008-9213-4
40. Mikulkova Z, Praksova P, Stourac P et al. Numerical defects in CD8+CD28- T-suppressor lymphocyte population in patients with type 1 diabetes mellitus and multiple sclerosis. *Cell Immunol* 2010; 262:75–9. https://doi.org/10.1016/j.cellimm.2010.02.002
41. van der Houwen TB, van Hagen PM, Timmermans WMC et al. Chronic signs of memory B cell activation in patients with Behcet's disease are partially restored by anti-tumour necrosis factor treatment. *Rheumatology (Oxford)* 2017; 56:134–44. https://doi.org/10.1093/rheumatology/kew366
42. Feng M, Zhou S, Liu T et al. Association between interleukin 35 gene single nucleotide polymorphisms and the uveitis immune status in a Chinese han population. *Front Immunol* 2021; 12:758554. https://doi.org/10.3389/fimmu.2021.758554
43. Ji X, Wu L, Marion T et al. Lipid metabolism in regulation of B cell development and autoimmunity. *Cytokine Growth Factor Rev* 2023; 73:40–51. https://doi.org/10.1016/j.cytogfr.2023.06.008
44. Wang Z, Lu Z, Lin S et al. Leucine-tRNA-synthase-2-expressing B cells contribute to colorectal cancer immunoevasion. *Immunity* 2022; 55:10671748–1081 e1068. https://doi.org/10.1016/j.immuni.2022.07.017

45. Tan J et al. Dietary carbohydrate, particularly glucose, drives B cell lymphopoiesis and function. *iScience* 2021; 24:102835. https://doi.org/10.1016/j.isci.2021.102835
46. Brookens SK, Cho SH, Paik Y et al. Plasma cell differentiation, antibody quality, and initial germinal center B cell population depend on glucose influx rate. *J Immunol* 2024; 212:43–56. https://doi.org/10.4049/jimmunol.2200756
47. Shimizu J, Takai K, Fujiwara N et al. Excessive CD4+ T cells co-expressing interleukin-17 and interferon-γ in patients with Behçet's disease. *Clin Exp Immunol* 2012; 168:68–74. https://doi.org/10.1111/j.1365-2249.2011.04543.x
48. Yasuoka H, Yamaguchi Y, Mizuki N et al. Preferential activation of circulating CD8+ and gammadelta T cells in patients with active Behcet's disease and HLA-B51. *Clin Exp Rheumatol* 2008; 26:S59–63.