

B Cell Activation, Differentiation, and Their Potential Molecular Mechanisms in Osteoarthritic Synovial Tissue

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Objective: The objective of this study was to characterize the activation and differentiation of B cells in the synovium of osteoarthritis (OA) and to explore the underlying molecular mechanisms.

Methods: Peripheral blood and synovial samples from OA patients at different stages were collected, and flow cytometry was employed to analyze the activation and differentiation of B cells. Immunofluorescence staining of joint synovium from OA mice at different stages was conducted to assess mice joint synovium B cell activation and differentiation. Co-culture experiments of synovial fibroblasts with B cells were performed to investigate the influence of synovial cells on B cell activation and differentiation. Finally, transcriptome analysis was utilized to identify potential key molecules and pathways.

Results: In OA patients, the infiltration, activation, and differentiation of B cells in synovium and peripheral blood exhibited distinct characteristics. Specifically, the proportion of activated CD86+ B cells and the differentiation marker HLA-DR+ increased with disease severity, whereas the proportion of the differentiation marker IgM decreased. The proportion of CD38+ B cells also decreased with increasing severity, although this change lacked statistical significance. Immunofluorescence staining of CD19+ and CD86+ cells in mice indicated increased expression with greater OA severity. Co-culture experiments demonstrated that OA synovial fibroblasts promoted B cell activation and differentiation, as evidenced by higher expression levels of CD86+ and HLA-DR+ in the OA group compared to controls. Additionally, the proportion of naive B cells decreased as disease severity progressed.

Conclusion: Synovial fibroblasts in OA have been shown to promote the differentiation and activation of B cells, indicating that B cells play a significant role in the pathogenesis of synovium inflammation in OA.

Keywords: osteoarthritis, B cells, inflammation, immune, transcriptome sequencing, synovium

Introduction

Osteoarthritis (OA) is a degenerative and progressive joint disease that affects approximately 500 million people globally.¹ Common risk factors for OA include advanced age, female gender, obesity, prior joint injuries, and chronic joint overuse, all of which contribute to disrupted load distribution.^{2,3} Inflammation plays a distinct role in the development and progression of OA across these phenotypes. Specific genetic alterations in proteins involved in inflammatory processes or cartilage matrix components may increase susceptibility to OA.⁴ While OA is a widespread condition, current treatment options are primarily aimed at alleviating its symptoms—pain and mobility issues.⁵ Pain management and physical therapy are the cornerstone approaches for primary treatment, as current interventions cannot halt or reverse the degenerative process. In advanced cases, joint replacement remains the final recourse for end-stage OA.⁶

OA is considered a whole-joint disease that affects not only the articular cartilage but also other tissues in and around the joint. Pathological features include degeneration of articular cartilage and ligaments, synovitis, subchondral bone

deformation, and osteophyte formation.⁷ Recent studies have reported that meniscal degeneration and tears can lead to OA, while the IFP contributes to OA progression by secreting various endocrine factors and inflammatory cytokines.^{8,9} While traditionally viewed as a non-inflammatory arthropathy, emerging evidence suggests a significant role of immune system components, particularly synovial inflammation, in the disease's progression.¹⁰ Among these immune components, B cells have garnered interest due to their ability to influence inflammatory responses and tissue remodeling through cytokine production and antibody secretion.¹¹

Influx of immune cells into the synovium, the tissue lining the joint capsule, is thought to mediate cartilage degradation by producing inflammatory mediators.¹² Recently, the mechanical behavior of IFP has been reported showing its involvement in worsening OA disease. In particular, IFP is smaller in end-stage OA compared to patients with knee trauma, thus supporting the role of this tissue changes in OA disease.^{13,14} These mediators promote the production of matrix-degrading enzymes in cartilage and reduce its synthetic activity.¹⁵ Furthermore, the synovium contributes to OA-related pain by promoting neurogenic inflammation mediated by neuropeptides like substance.^{15,16} Although traditionally considered a non-inflammatory joint disease, emerging evidence highlights the role of immune system components, particularly synovitis, in OA progression. Among these immune components, B cells have garnered interest due to their ability to influence inflammatory responses and tissue remodeling through cytokine production and antibody secretion.¹⁷

Synovial fibroblasts are key players in OA pathogenesis. They foster the inflammatory environment of the disease by secreting pro-inflammatory cytokines, chemokines, and extracellular matrix-degrading enzymes, collectively driving cartilage erosion and joint destruction.¹⁸ Additionally, synovial fibroblasts in OA may adopt a unique phenotype, rendering their inflammatory and catabolic functions persistent.¹⁹ Co-culturing B cells with synovial fibroblasts offers a complex in vitro model to explore the intercellular interactions that may exacerbate OA pathology.²⁰ Such studies are essential for elucidating the role of B cells in synovial fibroblast activation and the overall intra-articular inflammatory environment of OA. Understanding these interactions could unveil novel therapeutic targets to modulate the immune component of OA, providing potential interventions beyond symptom management that could alter the disease course.

Although in vivo studies have traditionally been conducted to validate the relevance of these interactions, there is a growing interest in replacing in vivo models with in vitro approaches, particularly in light of societal demands to reduce animal usage. Several types of in vitro models are available, and co-culture systems, which involve different cells or tissues, are particularly well-suited for investigating the role of joint tissue interactions in the pathophysiology of OA.²¹ Understanding the connections between different cell types and joint structures could further enhance our comprehension of OA development and progression. Consequently, therapies developed for patients with joint diseases will benefit from this improved understanding.

This study explores the dynamic interactions between B cells and synovial fibroblasts, using transcriptome sequencing to elucidate their roles in sustaining or potentially exacerbating the inflammatory processes in osteoarthritis. By investigating these interactions, the study aims to reveal how they influence OA pathogenesis, potentially providing new therapeutic strategies.

Materials and Methods

Clinical Synovial Sample Collection

Synovial samples undergoing total knee arthroplasty and anterior cruciate ligament injury were collected at the Department of Orthopedics, The First People's Hospital of Hefei. These samples were categorized into mild (grades 1–2) and severe (grades 3–4) groups based on radiographic Kellgren-Lawrence (KL) grading ([Figure S1](#)).²² This experiment complies with the Declaration of Helsinki and has been approved by the Ethics Committee of the Third Affiliated Hospital of Anhui Medical University (grant ID: 2023–021-01). Informed consent was obtained from all patients. For each patient, 200 mg of synovial tissue was minced and placed into 1640 medium (Gibco USA Cat no: C11875500BT) containing 10% fetal bovine serum (Gibco AUS Cat no: 10099141C). Type IV collagenase (Worthington USA Cat no: LS004188) was added at a concentration of 100 mg/mL, and the mixture was digested on a shaker at 37°C for 2 hours. The digested tissue was then filtered through a 200-mesh filter to retain the liquid, which was centrifuged to collect the cell pellet. Lymphocytes were

isolated from the cell pellet using Percoll (Cytiva GE Life USA Cat no:17089101) density gradient centrifugation with varying concentrations of Percoll, in preparation for flow cytometry analysis.

Animal Studies

All animal experiments were approved by the Animal Welfare and Ethics Committee of Anhui Medical University. Mice were housed at 23–25°C under a 12-hour light/dark cycle, with free access to food and water, in accordance with the guidelines outlined in the Laboratory Animal Science Guidelines of Anhui Medical University. Male C57BL/6 mice (8 months old) underwent destabilization of the medial meniscus (DMM) surgery on the right knee to induce an OA model. During the DMM surgery, the joint capsule was opened under anesthesia, and the medial meniscotibial ligament was transected to destabilize the meniscus without injuring other tissues. Synovial samples were collected at 4, 8 and 12 weeks post-surgery to obtain early, middle, and end-stage OA synovial samples.

Immunofluorescence Staining

OA model mice were euthanized, and cold PBS (Biosharp China Cat no: BL302A) perfusion was performed, followed by fixation in 4% paraformaldehyde (Servicebio China Cat no: G1101-500ML) for 2 days. The synovial tissues were sectioned into 50 µm slices and blocked with 0.1 M PBS containing 5% fetal bovine serum and 0.3% Triton X-100 (Servicebio China Cat no:GC204003-100mL) at room temperature for 1 hour. After washing, the sections were incubated overnight at 4°C with the following primary antibodies: anti-CD19 (Proteintech China Cat no: 27949-1-AP) and anti-CD86 (Proteintech China Cat no: 30,691-1-AP). The sections were then washed and incubated with Alexa 594-conjugated secondary (Biolegend USA Cat no: 405240) antibodies or Alexa 488-conjugated antibodies (Biolegend USA Cat no: 405235) at room temperature for 2 hours. After thorough washing, cell nuclei were stained with DAPI (Biolegend USA Cat no: 422801). All sections were imaged using a TCS SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany).

Synovial Fibroblast Preparation

After collecting knee joint specimens from OA patients, the synovial tissue samples were immediately isolated and prepared for cell culture. Briefly, the excised synovial tissue was cut into small pieces after soaking in PBS and arranged neatly along the sidewall of a cell culture flask. The bottom of the flask was filled with DMEM medium (Gibco USA Cat no: 62247) containing 20% FBS. The flask was placed vertically in an incubator, and after 8 hours, it was laid flat to allow the tissue to be immersed in the medium. One week later, synovial fibroblasts gradually migrated out from the synovium tissue. Synovial fibroblasts of passages 3–8 were used for experiments.

Stimulation of OA Synovial Fibroblasts

Approximately 10,000 synovial fibroblasts were seeded into a 6-well plate and grown for 2 days. Synovial fibroblasts were stimulated with IL-1β (10 ng/mL) (MCE USA Cat no: HY-P7028) in DMEM medium containing 10% FBS for 24 hours to simulate the OA environment, referred to as the experimental group, whereas the control group received an equal volume of PBS.^{7,23} The supernatant was then replaced and further cultured for 24 hours, after which the supernatants were collected for co-culture experiments ([Figure S2](#)).

Isolation of Naïve B Cells

Peripheral blood was collected from healthy volunteers, and PBMCs were obtained by gradient centrifugation using Human Lymphocyte Separation Medium (Dakewe China Cat no: 7111011). Naïve B cells were then isolated from PBMCs using the Human Naïve B Cell Isolation Kit (Stemcell Canada Cat no:17254) according to the manufacturer's instructions and subsequently cultured.

Co-Culture of Synovial Fibroblasts With Human Naïve B Cells

Briefly, 20,000 isolated human naïve B cells were seeded into a 24-well plate, and the collected supernatants from the stimulated and control groups were added for indirect co-culture experiments. The cells were cultured for 4 days, after which the differentiation and activation levels of B cells were assessed using flow cytometry ([Figure S2](#)).

Transcriptome Sequencing Analysis

All volunteers provided written informed consent, and all samples were anonymized. Blood samples were collected in the morning from fasting participants, with the same personnel involved in the experimental process to minimize variability. The samples were used for microarray transcriptomic analysis. On the fourth day of co-culture, RNA was extracted from B cells using TRIzol reagent (Invitrogen USA Cat no: 15596018CN). Total RNA was quantified using the NanoDrop ND-1000 spectrophotometer, and RNA-Seq libraries were constructed using 1–2 µg of total RNA. mRNA was enriched using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) following the manufacturer's instructions. Library construction was performed using the KAPA Stranded RNA-Seq Library Preparation Kit (Illumina). The sequencing libraries were evaluated using an Agilent 2100 Bioanalyzer with the Agilent DNA 1000 Kit (Agilent, Part #5067-1504). Sequencing was conducted on the Illumina HiSeq 4000 platform with a 150 bp paired-end read length. After quality control, raw sequencing data were aligned to the mouse genome (GRCm38) using the Hisat2 software. Differentially expressed genes were identified based on a fold change ≥ 1.5 and a p-value ≤ 0.05 . Cluster analysis was performed using Cluster 3.0 software, while gene ontology (GO) biological process analysis was conducted via DAVID. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was also performed to explore potential biological pathways.

Flow Cytometry

Flow cytometry was used to determine the proportions of various B cell types in peripheral blood synovial tissue and co-culture cells. Cell suspensions were counted and resuspended in 100 µL staining buffer, and incubated with monoclonal antibodies in the dark at 4°C for 30 minutes. Our flow cytometry staining strategy consisted of the following monoclonal antibodies conjugated to fluorescent dyes: anti-CD24-Alexa-Fluor700 (eBioscience USA Cat no: A15821), anti-HLA-DR-eFluor450 (eBioscience USA Cat no: MHLDR28), anti-CD27-APC (eBioscience USA Cat no: MA1-19761), anti-CD19-APC-Cyanine7 (eBioscience USA Cat no: A15429), anti-CD45-eFluor506 (eBioscience USA Cat no: 69045824), anti-IgM-PE (eBioscience USA Cat no: MA1-10381), anti-CD38-PE-Cyanine7 (eBioscience USA Cat no: 25-0381-82), and anti-CD86-FITC (eBioscience USA Cat no: MA1-10300). CD45 and CD19 were used to identify B cells. CD86+ cells were defined as activated B cells. After washing and resuspension, samples were analyzed using BD FACSDiva software on a BD FACSCanto II flow cytometer.

Statistical Analyses

All data comparisons will first undergo analysis for homogeneity of variance, independence, and normality. If these conditions are met, Student's *t*-test will be applied; otherwise, the rank-sum test will be used. All calculations were performed using the GraphPad Prism 9.5 program.

Result

In our initial flow cytometry analysis of synovial clinical samples, given the diverse cell types present in the synovium, we first identified the CD45-positive cells. Subsequently, we gated the CD19-positive population within the CD45-positive group to isolate B cells, followed by gating for various B cell markers. The gating strategy is illustrated in [Figure 1](#).

An Imbalance in the Ratio of Elevated B Cells and Activated B Cells in Human Osteoarthritic Synovial Tissue and Peripheral Blood

In our study, we observed a significant imbalance in the proportion of elevated B cells and activated B cells between the synovial tissue and peripheral blood of OA patients. After categorizing the samples into mild and severe groups based on imaging, we observed that B cell infiltration in the synovium of severe OA samples was significantly higher than that in mild OA samples, with statistical significance ([Figure 2A](#) and [B](#)). CD86 is a critical marker of B cell activation, the

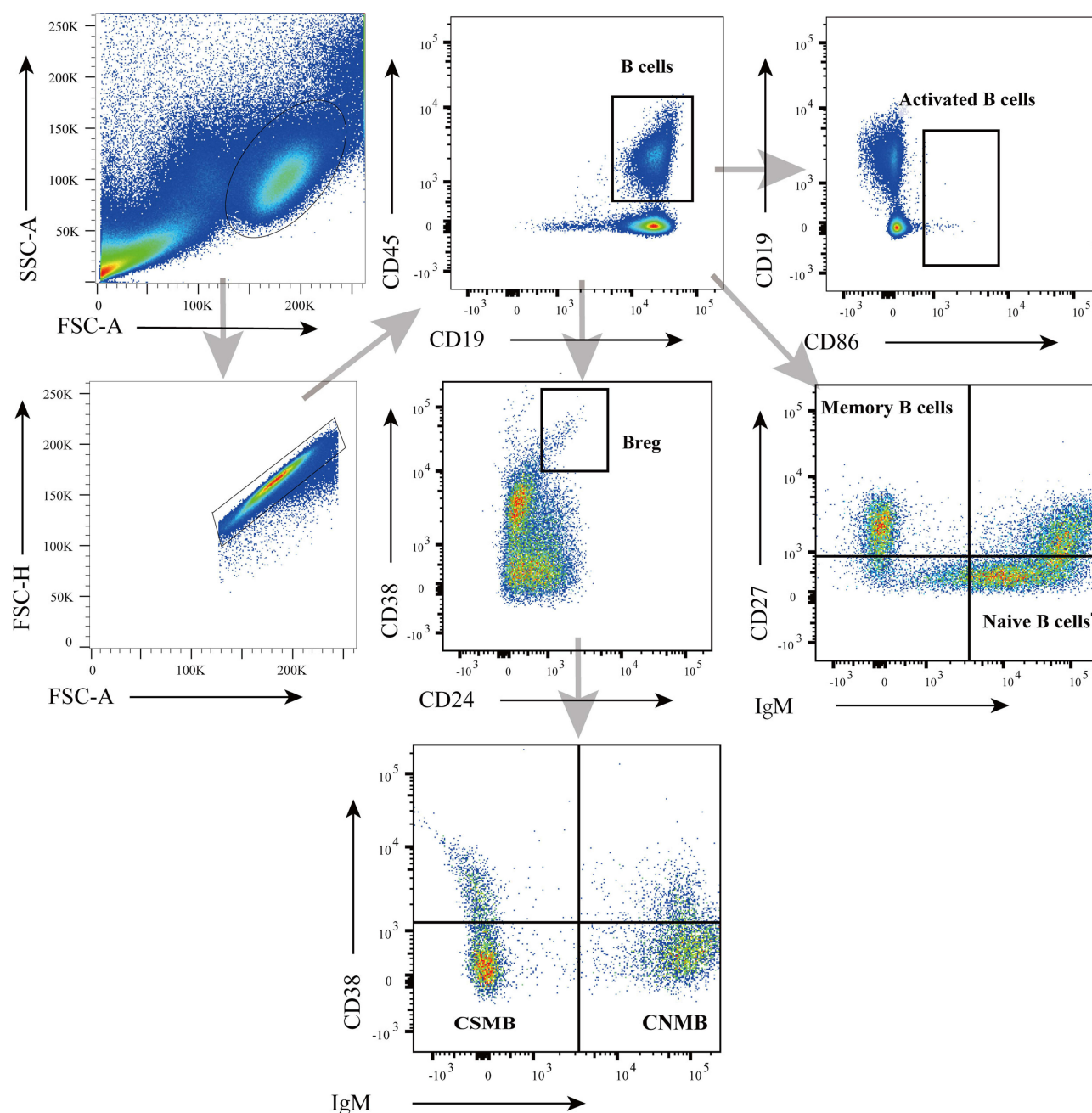


Figure 1 Gating strategy for B cell subsets. This gating strategy applies to peripheral blood, synovial tissue, and B cells from OA patients. Briefly, lymphocyte populations were first gated based on forward/side scatter (FSC/SSC) profiles, excluding debris. Subsequently, different B cell subsets were identified using specific markers to define each subset.

proportion of CD86-positive cells was notably elevated in the severe group compared to the mild group, highlighting a marked increase in B cell activation with disease progression (Figure 2C and D). CD38, a marker of B cell differentiation and activation, typically plays a key role in immune responses. While the proportion of CD38-positive cells did not exhibit a statistically significant difference between the two groups, it is noteworthy that the severe group had a relatively lower percentage (Figure 2E and F). Additionally, HLA-DR expression, a marker of antigen-presenting capability in B cells, was considerably higher in the severe group (Figure 2G and H). In contrast, IgM-positive cell proportions were higher in the mild group (Figure 2I and J).

Flow cytometry analysis of peripheral blood revealed an increase in B cell infiltration in the severe group compared to the mild group, although the difference was not as pronounced as observed in the synovial tissue (Figure 3A and B).

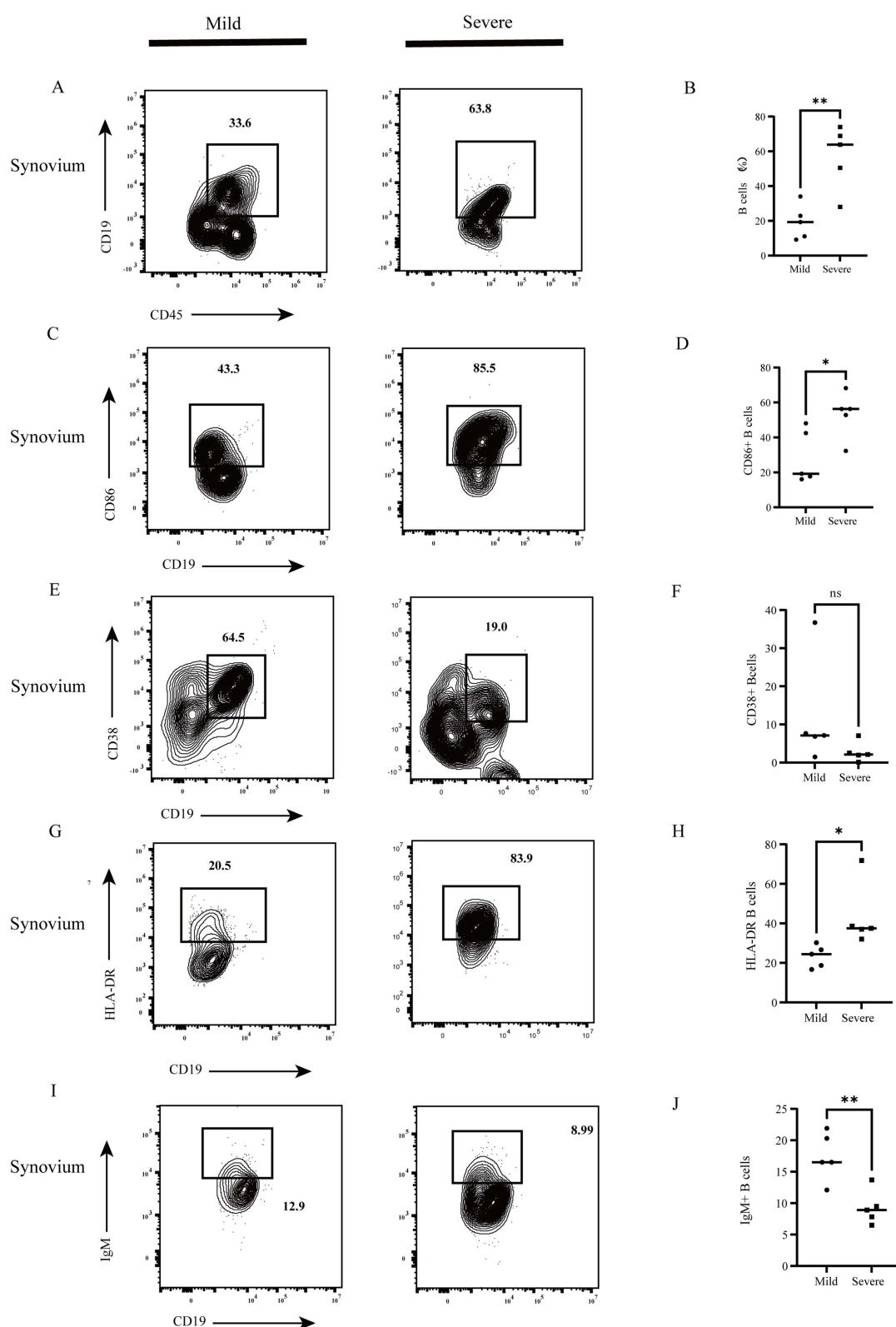


Figure 2 Analysis of B Cell Subsets in OA Synovium: Flow cytometry was used to detect the proportions of (A-B) total B cells, (C-D) CD86+ B cells, (E-F) CD38+ B cells, (G-H) HLADR+ B cells, and (I-J) IgM+ B cells. (* $p < 0.05$, ** $p < 0.01$, ns, not significant).

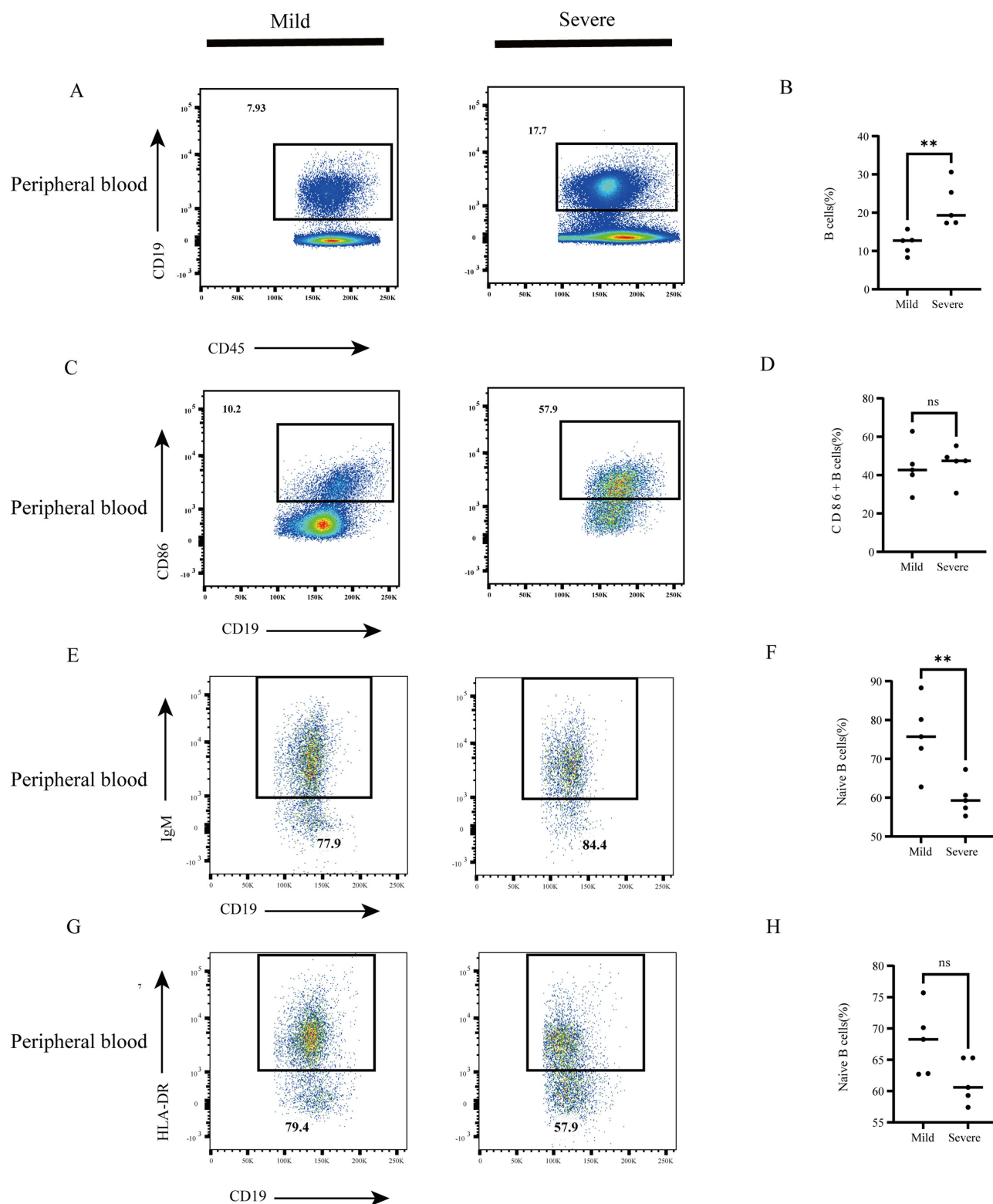


Figure 3 Analysis of B cell subsets in the peripheral blood of OA patients: Flow cytometry was performed to detect the proportions of (A-B) total B cells, (C-D) memory B cells, (E-F) naive B cells, and (G-H) CD38+ B cells. (*p < 0.05, **p < 0.01, ns, not significant).

Despite the overall rise in B cell infiltration, the proportion of CD38-positive cells and Memory B cells showed no significant variation between the two groups (Figure 3C, D, G and H). Additionally, the proportion of naive B cells in peripheral blood was higher in the mild group than in the severe group, this is similar to what we observed in the synovial tissue (Figure 3E and F).

The Landscape of B Cell Activation and Differentiation in Mouse Synovium Changes With the Severity of OA

In the OA mouse model, mice were grouped into early, middle, and late stages according to the progression of the disease. Immunofluorescence analysis was conducted to evaluate the expression levels of CD19 and CD86 within the synovium of these mice. The results showed a clear trend of increasing CD19 and CD86 expression as the severity of OA advanced. In the early-stage group, CD19 expression was relatively low, indicating limited B cell infiltration. By the late stage, the expression of CD19 was markedly higher. Similarly, the expression of CD86, a key marker of B cell activation, was low in the early-stage group but significantly increased in the middle and late stages. By the late stage, CD86 expression reached its highest levels, showing extensive activation of B cells in the severely affected synovial tissue. The results revealed that, similar to human synovial samples, B cell infiltration and activation levels were significantly higher in the severe OA group compared to the mild group (Figure 4).

Synovial Fibroblasts Promote B Cell Differentiation and Activation

In the indirect co-culture experiment of synovial fibroblasts and B cells, two groups were analyzed: the IL-1 β stimulated group and the control group. The results showed that B cell differentiation and activation were significantly higher in the stimulated group compared to the non-stimulated group. Specifically, the proportions of CD27-positive (Figure 5A and B), CD86-positive (Figure 5C and D), and HLA-DR-positive B cell populations were markedly elevated in the stimulated group, with statistically significant differences observed (Figure 5G and H). Conversely, the proportion of Naive B cells was higher in the control group, indicating that in the absence of stimulation, B cells maintained a less activated state (Figure 5E and F). Notably, there was no statistically significant difference in the percentage of CD38-positive B cells between the two groups, suggesting that CD38 expression was not significantly affected by the stimulation conditions used in this study (Figure 5I and J).

Transcriptome Sequencing

We performed transcriptome sequencing on the co-cultured samples from day four, and the volcano plot revealed a total of 204 upregulated differentially expressed genes (DEGs) and 1429 downregulated DEGs (Figure 6A). A heatmap illustrated the

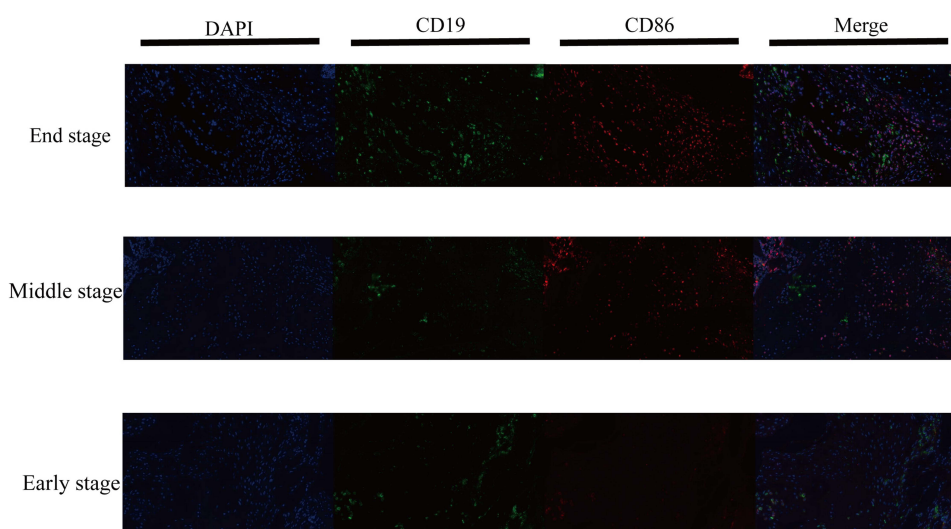


Figure 4 Immunofluorescence staining of mouse synovium: CD19 (green) and CD86 (red) were used to label B cells, while DAPI (blue) was used for nuclear staining.

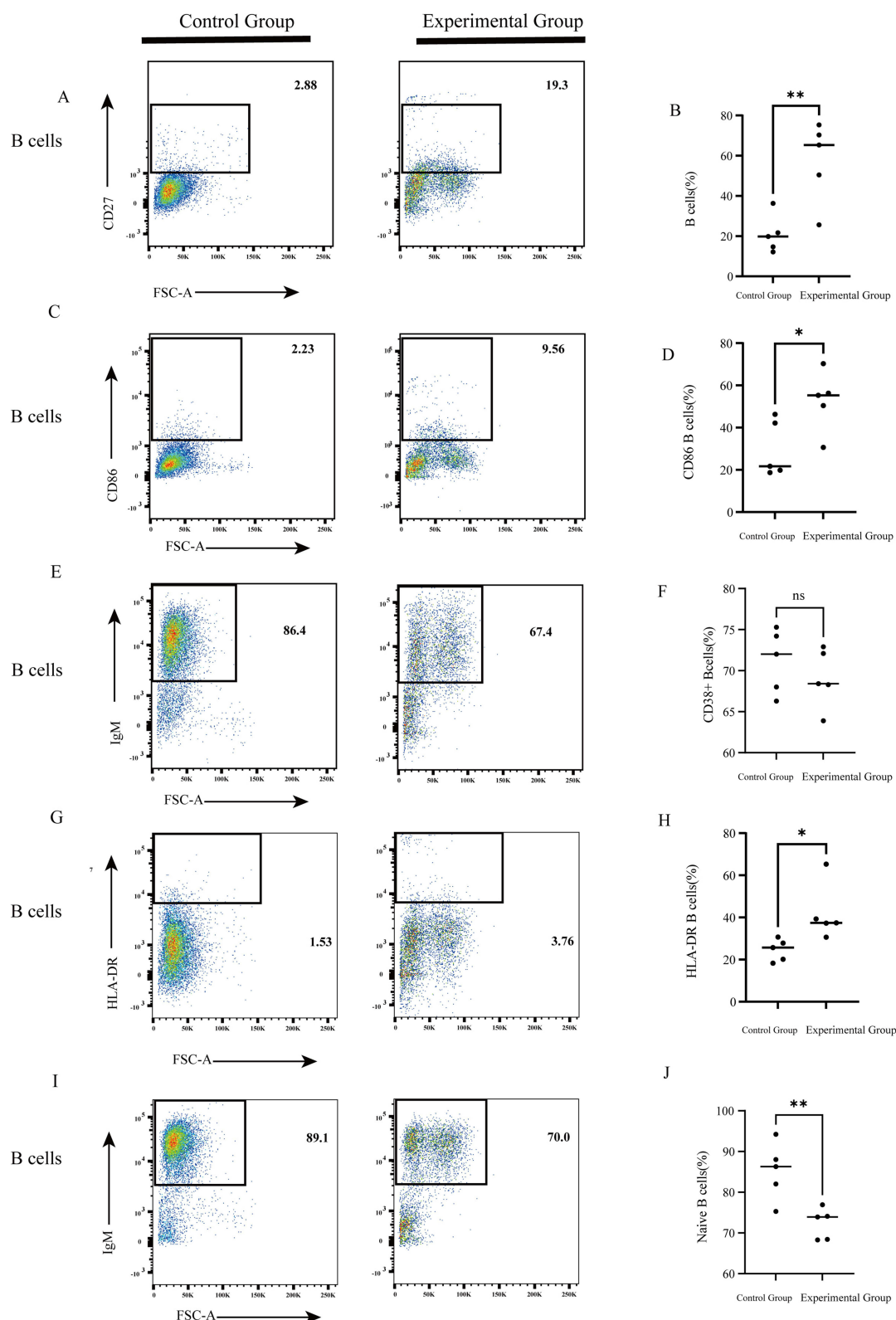


Figure 5 B Cell Subset Analysis in Co-Culture Experiments: Flow cytometry was used to detect the proportions of (A-B) total B cells, (C-D) CD86+ B cells, (E-F) CD38+ B cells, (G-H) HLA-DR+ B cells, and (I-J) Naive B cells. (* $p < 0.05$, ** $p < 0.01$, ns, not significant).

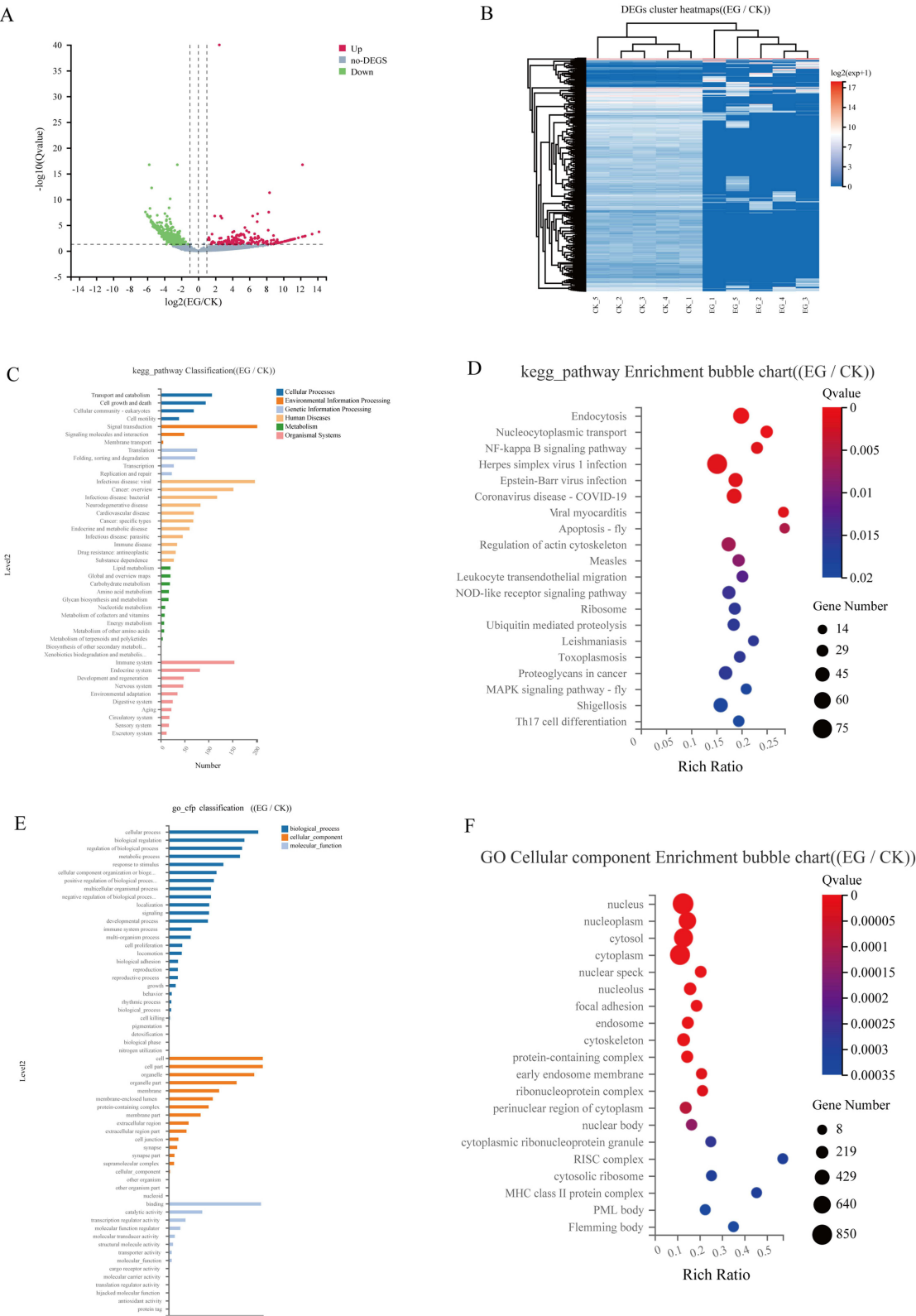


Figure 6 Transcriptomic Analysis of B Cells. The experimental group is labeled as EG, and the control group is labeled as CK. **(A)** Volcano Plot **(B)** Heatmap of Differentially Expressed Genes. KEGG Enrichment Analysis **(C)** Bar Chart **(D)** Bubble Chart GO Enrichment Analysis **(E)** Bar Chart **(F)** Bubble Chart.

differential expression levels of genes between the two groups (Figure 6B). KEGG pathway enrichment analysis revealed that the supernatant of synovial fibroblasts stimulated with IL-1 β significantly enriched several pathways in Naïve B cells, among which endocytosis, NF-kappa B signaling pathway, and MAPK signaling pathway exhibited particularly high levels of enrichment (Figure 6C and D). Additionally, GO enrichment analysis highlighted significant associations with the nucleus and MHC class II protein pathways. (Figure 6E and F).

Discussion

OA is the most common form of joint disease, affecting millions of people worldwide. It is particularly prevalent in the aging population, with estimates suggesting that more than 10% of individuals over the age of 60 are affected by symptomatic OA, primarily in weight-bearing joints such as the knees and hips.²⁴ The risk factors for OA include age, obesity, joint injury, and repetitive joint stress, making it a significant cause of disability and reduced quality of life globally²⁵. The pathogenesis of OA has traditionally been viewed as a degenerative process involving the breakdown of articular cartilage.²⁶ However, recent research has shifted this paradigm, highlighting the role of inflammation and the involvement of multiple joint tissues, including the synovium, subchondral bone, and surrounding ligaments.²⁷ Similarly, the IFP produces cytokines, adipokines, and growth factors, which influence OA progression through paracrine mechanisms, and the meniscus, when dysfunctional or damaged, ultimately contributes to the development of OA.^{8,9} Synovitis, in particular, has been identified as a key player in OA progression, contributing to cartilage degradation through the production of inflammatory mediators and matrix-degrading enzymes. The complex interplay between mechanical stress, immune responses, and metabolic factors is now recognized as central to OA development and progression.

In the context of OA, immune cells, particularly B cells, are gaining attention for their role in joint inflammation. B cells are traditionally known for their role in adaptive immunity, producing antibodies and contributing to immune memory.²⁸ However, in OA, B cells have been found to infiltrate the synovium, where they may exacerbate inflammation and tissue damage.²⁹ Studies have demonstrated increased B cell presence in OA synovium, and their activation is associated with the production of pro-inflammatory cytokines such as IL-6 and TNF- α , which contribute to the inflammatory milieu within the joint.³⁰ Moreover, B cells can act as antigen-presenting cells, further amplifying immune responses and driving synovitis. This suggests that B cells not only participate in systemic immune responses but also play a critical role in the local joint environment, influencing the chronic inflammatory processes that characterize OA.³¹ Understanding the precise mechanisms by which B cells contribute to OA pathogenesis may provide new therapeutic targets aimed at modulating B cell activity to alleviate joint inflammation and slow disease progression.

Synovial fibroblasts are key effector cells in the inflamed synovium of OA patients, producing cytokines, chemokines, and matrix-degrading enzymes that perpetuate inflammation and cartilage destruction.³² Emerging evidence suggests that synovial fibroblasts can directly interact with immune cells, including B cells, to modulate the local immune response.³³ These interactions are mediated through cell-cell contact and the release of soluble factors, such as IL-6 and BAFF (B cell-activating factor), which promote B cell survival and activation. The result is a more aggressive inflammatory response within the joint, characterized by increased production of autoantibodies and further synovial hyperplasia.³⁴ This reciprocal relationship between synovial fibroblasts and B cells underscores the complexity of the OA joint environment, where immune cells and resident stromal cells collaborate to drive disease progression. Targeting this interaction may represent a novel approach to mitigating synovitis and halting the destructive processes in OA.

We found that the percentage of B cells in OA synovial tissue was significantly higher compared to peripheral blood, this suggested that the synovial tissue was a key site for B cell infiltration and local activation. B cells accumulated within the synovium and contributed to the inflammatory response, potentially exacerbating synovitis and joint damage through the secretion of antibodies and pro-inflammatory cytokines such as TNF- α and IL-6.³⁵ This localized immune response differed from the role of B cells in peripheral blood, where they were primarily involved in systemic immune surveillance. In contrast, synovial B cells may have acted as local drivers of inflammation in OA.

CD86 is a crucial co-stimulatory molecule expressed on B cells that plays a key role in their activation. It binds to the CD28 receptor on T cells, providing a necessary second signal in addition to the antigen-specific signal for full T-cell activation. This interaction promotes T-cell proliferation and cytokine production, which in turn influences B cell activation.^{36,37} Regarding OA and B cells, increased expression of CD86 has been associated with enhanced B cell

activation and may contribute to the inflammatory processes observed in OA.³⁸ Studies have suggested that B cells expressing CD86 can promote the inflammatory milieu by interacting with T cells, exacerbating synovitis and contributing to cartilage degradation.³⁹ The proportion of activated B cells, was higher in synovial tissue than in peripheral blood. This imbalance suggests that, while B cells are present systemically, they become more active and concentrated within the inflamed synovium, potentially contributing to the local inflammatory milieu.⁴⁰ These findings underscore the role of B cells in the pathophysiology of OA, particularly in synovial inflammation, and suggest that B cell activation within the synovium may be a key driver of disease progression.

HLA-DR is a major histocompatibility complex (MHC) class II molecule that plays a crucial role in antigen presentation, which is essential for B cell activation and differentiation.⁴¹ B cells express HLA-DR to present processed antigens to CD4⁺ helper T cells, leading to T cell activation. This interaction provides critical signals that promote B cell differentiation into antibody-producing plasma cells and memory B cells. Without effective antigen presentation via HLA-DR, B cell responses can be impaired, leading to reduced antibody production and a weakened immune response.⁴² In OA and other inflammatory diseases, increased expression of HLA-DR on B cells has been associated with their heightened activation and potential involvement in local inflammatory processes. While specific studies on HLA-DR in OA are limited, research in RA has demonstrated that elevated levels of HLA-DR on B cells correlate with disease activity and synovial inflammation, suggesting a similar mechanism may be relevant to OA.⁴³ HLA-DR expression, was considerably higher in the severe group. This suggests an increased involvement of B cells in antigen presentation as the disease advances, possibly contributing to the heightened inflammatory environment in OA. In both our co-culture experiments and clinical synovial tissue samples, HLA-DR expression consistently increased with the severity of OA. This suggests that as OA progresses, B cells become more actively involved in antigen presentation, which in turn exacerbates the inflammatory environment within the joint.

IgM is the first antibody produced during the initial stages of an immune response, particularly by naïve B cells. It is crucial for initiating immune responses to pathogens. IgM can activate the complement system, which enhances the ability to clear pathogens and damaged cells.^{44,45} Naïve B cells are B cells that have not yet encountered an antigen. They primarily express IgM and IgD on their surface. Upon encountering an antigen, they undergo differentiation into either plasma cells (which secrete antibodies) or memory B cells (which provide long-term immunity).⁴⁶ The proportion of naïve B cells in a population can indicate the readiness of the immune system to respond to new antigens. A decrease in naïve B cells, as seen in some chronic inflammatory conditions, may suggest that the immune system has been persistently activated, leading to differentiation into more mature B cell subsets.⁴⁷ IgM-positive cell proportions were higher in the mild group, indicating that naïve or less differentiated B cells were more prevalent in early-stage disease, while more advanced disease stages may be characterized by a shift toward memory or activated B cells. In the co-culture non-stimulated group and the mild synovitis group, the proportion of IgM-positive cells and naïve B cells was notably higher. This suggests that immature or less differentiated B cells are more prevalent during the early stages of the disease, while later stages may be characterized by a shift toward memory or activated B cells. These findings serve as evidence that synovial fibroblasts play a role in promoting B cell differentiation. Overall, the co-culture system clearly demonstrated that synovial fibroblasts facilitate both the activation and differentiation of B cells, indicating a direct interaction between these cell types in the context of OA-related inflammation.

The sequencing results of B cells showed that, in the KEGG enrichment analysis, the NF- κ B signaling pathway ranked highly among the enriched inflammatory pathways. NF- κ B is a key signaling pathway in inflammation and immune regulation, playing a decisive role in B cell activation, differentiation, and survival.⁴⁸ Studies have reported that B cell activation begins with the stimulation of B cell-activating factor (BAFF), which regulates B cell activation and differentiation through the activation of both classical and non-classical NF- κ B signaling pathways.⁴⁸ Our sequencing results showed that the expression of BAFF receptor (BAFFR) was upregulated. This finding further supports the hypothesis that the abnormal activation and differentiation of B cells in OA synovium may be mediated by BAFF and other cytokines secreted by the synovium, which activate the NF- κ B signaling pathway. Endocytosis is a common cellular uptake pathway that plays a critical role in immune responses by facilitating antigen uptake, processing, and presentation to activate the immune system.⁴⁹ In B cell activation and differentiation, endocytosis plays an indispensable role. Upon stimulation by foreign

antigens, B cell receptors (BCRs) can initiate endocytosis, process and present antigens, trigger cascade reactions, and activate downstream signaling pathways, thereby promoting the differentiation and activation of B cells.⁵⁰

One limitation of this study is the relatively small sample size, which may reduce the generalizability of the findings to the broader OA population. Another limitation is the focus on specific markers like CD86, IgM and HLA-DR, which might not capture the full complexity of B cell activation and differentiation in OA. Moreover, the *in vitro* co-culture experiments may not fully replicate the *in vivo* environment, potentially limiting the applicability of the results to clinical settings. Future studies with larger cohorts, longitudinal designs, and more comprehensive phenotyping are needed to further validate and expand upon these findings.

The results of this study confirmed the significant impact of OA synovial fibroblasts on B cell differentiation and activation, leading to the conclusion that B cells may be involved in the pathogenesis of synovial inflammation in OA. Understanding the importance of synovial modulation of B cell differentiation and activation could pave the way for new preventive and therapeutic strategies for OA by targeting immune responses.

Conclusion

This study highlights the distinct patterns of B cell activation and differentiation in the synovium and peripheral blood of OA patients. The progression of OA is associated with increased proportions of activated B cells (CD86+) and differentiated B cells (HLA-DR+), accompanied by a decrease in IgM+ naive B cells. Immunofluorescence analysis of mouse synovium further validated the correlation between B cell activation and OA severity. Co-culture experiments confirmed that OA synovial play a critical role in promoting B cell activation and differentiation. These findings underscore the involvement of B cells in the inflammatory microenvironment of OA and suggest that targeting the interaction between synovial fibroblasts and B cells may provide novel therapeutic strategies for OA management.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

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We thank all the people who offer help for this study. Co-first authors: Peizhi Lu, Ya Li contributed equally to this paper. Co-Correspondence authors: Bizhi Tu, Rende Ning contributed equally to this paper.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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