Article

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Autophagy-Mediated B Lymphocyte Dysfunction in the Pathogenesis of IgG4-Related Disease

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Background: Immunoglobulin G4-related disease (IgG4-RD) is a multisystem immune disorder characterized by fibroinflammatory lesions involving multiple organs. B cells play a pivotal role in IgG4-RD pathogenesis, and autophagy substantially influences their functional capacity. This study aimed to evaluate the role of B-cell autophagy in IgG4-RD.
Methods: Single-cell sequencing data were analyzed. Autophagy was assessed by flow cytometry with CYTO-ID® Autophagy Detection Kit 2.0 (CytoID) and by measuring autophagy-related proteins in peripheral blood mononuclear cell (PBMC) B cells from IgG4-RD patients and healthy controls (HCs) via polymerase chain reaction (PCR) and western blotting. Autophagy in B cells from IgG4-RD patients and HCs was inhibited, and B-cell activity was evaluated using Enzyme-linked immunoassay (ELISA) and flow cytometry.
Results: Altered autophagy levels and extracellular signal-regulated kinase (ERK) pathway activity were observed in total B cells, as well as in naïve B cells and plasmablasts. Autophagy in B cells was enhanced, as indicated by greater autophagosome formation and higher levels of autophagy-related proteins ($p < 0.05$). ERK phosphorylation was markedly reduced in IgG4-RD compared to HCs ($p < 0.05$). Following autophagy inhibition, B-cell metabolism, proliferation, antibody production, and cytokine secretion were significantly decreased in IgG4-RD ($p < 0.05$). Cell differentiation was similarly affected, with reduced memory B-cell and plasma cell proportions but elevated regulatory B-cell proportions ($p < 0.05$), whereas the reduction of memory B cells in HCs was not significant.
Conclusion: IgG4-RD patients exhibited defective B-cell autophagy and elevated autophagy levels associated with multiple serological and hematological markers, alongside impaired autophagy flux. Significantly reduced ERK1/2 phosphorylation was uniquely observed among signaling pathways, suggesting potential autophagy-apoptosis interactions. Upon autophagy inhibition, B cells exhibited a broad functional decline and anti-inflammatory features, highlighting an autophagy-B-cell activity relationship that may be valuable for disease monitoring.

Keywords: immunoglobulin G4-related disease; B cells; autophagy

Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a multisystem immune-mediated fibroinflammatory disorder with the potential to affect multiple organs [1,2]. Its characteristic histopathological features include $IgG4^+$ plasma cell-rich lymphoplasmacytic infiltrates, storiform fibrosis, and obliterative phlebitis [3]. In IgG4-RD, B cells exhibit abnormal metabolism, dysregulated antibody secretion, and increased production of profibrotic factors [4,5]. The clinical success of anti-Cluster of Differentiation (CD) 20 and anti-CD19 monoclonal antibody therapies in these patients underscores the central role of B cells in IgG4-RD development [6,7].

Autophagy, a conserved intracellular degradation process, is essential for maintaining B-cell metabolic homeostasis, supporting peripheral activation, sustaining memory cell survival, and facilitating antigen presentation [8]. B-cell autophagy is critical for lipopolysaccharide-stimulated humoral autoimmune responses [9], and its dysregulation has been implicated in multiple immune diseases. For example, in systemic lupus erythematosus (SLE), autophagic activity is elevated in both B cells and plasmablasts [10]. Toll-like receptor (TLR)-7-mediated autoimmune responses require intact B-cell autophagy, whereas autophagy deficiency reduces cytokine production [11]. In asthma-prone mice, impaired B-cell autophagy alleviates immunopathology, while interleukin 4 (IL-4) promotes B-cell autophagy to enhance cell survival and antigen

presentation [12]. Similarly, IL-17 promotes plasma cell differentiation through autophagy [13], and restoration of autophagy in the spinal cord via AG490 through the Janus Kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway exerts neuroprotective effects [14]. Conversely, inhibition of autophagy by deletion of transmembrane 9 superfamily member 1 (TM9SF1) inhibits B-cell differentiation into antibody-secreting plasma cells [15].

Single-cell sequencing has also revealed upregulation of the phosphatidylinositol-3-kinase (PI3K)/serine/threonine kinase (AKT) pathway in memory B cells, a change associated with autophagy [4]. In IgG4-related sialadenitis, acinar cells exhibit dysregulated autophagy, with extracellular signal-regulated kinase (ERK) 1/2 phosphorylation mediating tumor necrosis factor-α (TNFα)-induced autophagy suppression [16]. In lupus nephritis, demethylzeylasteral promotes autophagy and suppresses inflammation in podocytes, alleviating podocyte injury [17]. In type 1 diabetes, autophagy defects increase the vulnerability of β-cells to immune-mediated destruction [18]. In Graves' disease, thyrocyte autophagy is dysregulated, appearing inhibited in the early phase and activated in the late phase [19].

Although altered metabolism and function of B cells in IgG4-RD have been documented, the status of B-cell autophagy, a key pathway for regulating cellular metabolism and maintaining intracellular homeostasis, has not been investigated in IgG4-RD. This study aimed to characterize autophagy alterations and their underlying mechanisms in B cells from IgG4-RD patients. Furthermore, we examined the effects of abnormal autophagy on B-cell functions, including metabolism, proliferation, differentiation, and antibody production, by experimentally modulating B-cell autophagy.

Materials and Methods

Patients

Sixty-four treatment-naïve IgG4-RD patients, newly diagnosed according to the 2019 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria [1], were recruited. Exclusion criteria included malignancies, additional autoimmune disorders, current infections, and conditions that could mimic IgG4-RD. Detailed clinical characteristics of the patients are provided in the **Supplementary Table 1**. Sixty-four healthy controls (HCs) were recruited from a volunteer group, matched for gender with the IgG4-RD patients (**Supplementary Table 2**). Peripheral blood samples from patients and their matched HCs were collected within 4 hours between them. The IgG4-RD responder index (RI) was used as the measure of disease activity [20].

Enzyme-Linked Immunoassay (ELISA)

B-cell culture supernatants were harvested and stored at –20 °C for up to two weeks before use. Antibody and cytokine concentrations were measured using the following kits: IgG ELISA (E88-104, Bethyl Laboratories, Hamburg, Germany; and Multi Sciences, Hangzhou, China), IgG1 ELISA (EHIGG1, Invitrogen, Carlsbad, CA, USA), IgG4 ELISA (HUFI02581, AssayGenie, Dublin 2, Ireland), and IL-6 ELISA (EK106/2–96, Multi Sciences, Hangzhou, China). All kits were used following the manufacturers' instructions.

Flow Cytometry

The following antibodies were used: anti-CD19 (302208), anti-Ki-67 (350514), anti-CD24 (311117), anti-IgD (348207), anti-CD27 (356411), anti-CD38 (303533), and anti-ATG5 (847405) from Biolegend (San Diego, CA, USA), and anti-Microtubule-Associated Protein 1 Light Chain 3 (LC3B) antibody (PA5-22711) from Invitrogen (Carlsbad, CA, USA). All antibodies were diluted 1:50. Autophagy was assessed using the CYTO-ID® Autophagy Detection Kit 2.0 (ENZ-KIT175-0200, Enzo, Long Island, NY, USA) [21]. For cytokine profiling, the LEGENDplex™ Multi-Analyte Flow Assay Kit Human B Cell Panel (13-plex) with V-bottom plate (740527, BioLegend, San Diego, CA, USA) was employed. Samples were analyzed using a BD FACSAria II system (BD Biosciences, Franklin Lakes, NJ, USA), and data processed with FlowJo 10.8.1 software (FlowJo, Ashland, OR, USA).

Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were extracted using the standard Ficoll-Hypaque method. CD19+ B cells for RNA or protein extraction were enriched by positive selection with CD19 MicroBeads, human (130-050-301, Miltenyi Biotec, Bergisch Gladbach, North Rhine-Westphalia, Germany), yielding $\geq$98% purity as verified by flow cytometry (Fig. 2J). CD19+ B cells for cell culture were isolated by negative selection using the EasySep™ Human B Cell Isolation Kit (17954, STEMCELL Technologies, Vancouver, British Columbia, Canada), achieving $\geq$95% purity as verified by flow cytometry (Fig. 4E).

B-Cell Proliferation, Metabolism, Differentiation, and Antibody Measurement

Cell cultures were maintained in RPMI 1640 medium (11875093, Gibco, Grand Island, NE, USA) supplemented with 10% fetal calf serum (10099141C, Gibco, Grand Island, NY, USA, origin: Australia) and 100 U/mL penicillin/streptomycin (15140122, Gibco, Grand Island, NY, USA). B cell activation was induced using 5 µg/mL anti-human IgM (314502, BioLegend, San Diego, CA, USA), 500 ng/mL recombinant human sCD40L (310-02, PeproTech, Cranbury, NJ, USA), 100 ng/mL IL-4 (200-04, Pe-

proTech, Cranbury, NJ, USA), and 50 ng/mL IL-21 (200-21, PeproTech, Cranbury, NJ, USA). Autophagy inhibition in cultured B cells was achieved with 5 mmol/L 3-methyladenine (3-MA, HY-19312, MCE, Monmouth Junction, NJ, USA) and 20 µmol/L bafilomycin A1 (BafA1, HY-100558, MCE, Monmouth Junction, NJ, USA) [22]. BafA1 was dissolved in dimethyl sulfoxide (DMSO, D8371, Solarbio, Beijing, China) (<2.5‰ DMSO), and 3-MA was dissolved directly in the medium. On day 5, B cells were harvested for flow cytometric assessment of proliferation, metabolism, and differentiation, and supernatants were collected for ELISA quantification of IgG, IgG1, IgG4, and IL-6.

Cell metabolism was assessed using a Cell Counting Kit-8 (CCK-8, AQ308, Aoqing Biotechnology, Beijing, China). After adding CCK-8 reagent to the cell cultures, optical density at 450 nm was measured following a 2-hour incubation using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). All cells used in the experiment were confirmed free from mycoplasma contamination by polymerase chain reaction (PCR). All cells were collected from human peripheral blood.

Polymerase Chain Reaction (PCR)

B cells were isolated using the RNA-Quick Purification Kit (YISHAN Biotechnology, Shanghai, China). RNA was quantified and reverse-transcribed using PrimeScript™ RT Master Mix (Perfect Real Time, RR036A, TaKaRa, Kyoto, Japan) and amplified with TB Green® Premix Ex Taq™ II (RR820A, TaKaRa, Kyoto, Kyoto Prefecture, Japan) on a QuantStudio™ 7 Pro Real-Time PCR System (Applied Biosystems™, Carlsbad, CA, USA), following the manufacturer's instructions. Primers were synthesized by RuiBiotech (Beijing, China), with sequences provided in **Supplementary Table 3**. Relative mRNA expression levels were calculated as $= 2^{CT_{\beta\text{-actin}} - CT_{target}}$, where *β-actin* served as the internal control, and the target gene was *LC3B* or *ATG5*. Data were normalized before presentation.

Western Blotting

B cells were harvested from PBMCs or the culture system. Total protein was extracted using RIPA buffer (high, R0010, Solarbio, Beijing, China) containing protease and phosphatase inhibitors (HX1863/HX1864, Huaxingbio, Zhenjiang, China), and protein concentrations were determined using a BCA Assay Kit (Pierce Biotechnology, Rockford, IL, USA). The following antibodies were used for immunoblotting: LC3B (43566T), ATG5 (12994T), SQSTM1/p62 (8025T), Phospho-MAPK Family Antibody Sampler Kit (9910T), p38 (8690T), p44/42 (ERK1/2) (4695T), and SAPK/JNK (9252T) from Cell Signaling Technology (Danvers, MA, USA); Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (BE0023), goat anti-mouse IgG (H&L)-HRP conjugated (BE0102), and goat anti-rabbit IgG (H&L)-HRP conjugated (BE0101) from EASYBIO (Beijing, China). Primary antibodies were diluted 1:1000, and secondary antibodies 1:5000. Image acquisition and analysis were performed using a Chemiluminescent Imaging System (SH-Cute 523, SHST, Hangzhou, China). The optical density of each band was quantified using ImageJ 1.45d (NIH, Bethesda, MD, USA) with Java 1.8.0_345 (64-bit, Oracle, Austin, TX, USA). Merged chemiluminescent and marker images were presented. GAPDH was used as a loading control for quality assurance.

Statistical Analysis

All statistical analyses were conducted in RStudio (2024.04.2+764, Posit, Boston, MA, USA) with R 4.2.1 (AT&T BellLaboratories, Murray Hill, NJ, USA). Two-group comparisons were performed using the *t*-test or the Wilcoxon test, as appropriate. Correlations between variables were examined using Pearson's test for normally distributed data and Spearman's test for non-normal data. Statistical significance was defined as a two-tailed $p < 0.05$. Continuous variables were presented as mean ± standard error of the mean (SEM) for normally distributed data or as median and interquartile range (IQR) for non-normal data. Categorical variables were presented as counts and percentages.

Results

Single-Cell Sequencing Indicates Altered Autophagy in B Cells in IgG4-RD

Our previous single-cell sequencing analysis comparing IgG4-RD patients with HCs identified enrichment of the "PI3K-Akt signaling pathway" in memory-unswitched B cells and the "IL-17 signaling pathway" in plasma cells [4]. Further analysis of the single-cell sequencing results using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) revealed alterations in autophagy-related processed in naïve B cells and plasma cells (Fig. 1A). Gene Set Enrichment Analysis (GSEA) [23,24] demonstrated upregulation of a panel of autophagy-related genes in B cells from IgG4-RD patients, supporting the observation of altered autophagy in naïve B cells and plasma cells [25] (Fig. 1B). In parallel, the ERK signaling pathway was found to be upregulated in plasma cells (Fig. 1C). These findings indicate that autophagy is altered in B cells of IgG4-RD patients and suggest that ERK signaling may be involved in this alteration.

Autophagy Is Enhanced in IgG4-RD and Correlates With Clinical Parameters

Compared to HCs, IgG4-RD patients exhibited significantly elevated dye of CYTO-ID® Autophagy Detection Kit 2.0 (CytoID) mean fluorescence intensity (MFI) in total B cells and a higher proportion of CytoID$^+$ B cells in PBMC ($p < 0.01$, Fig. 2A,B). However, no significant

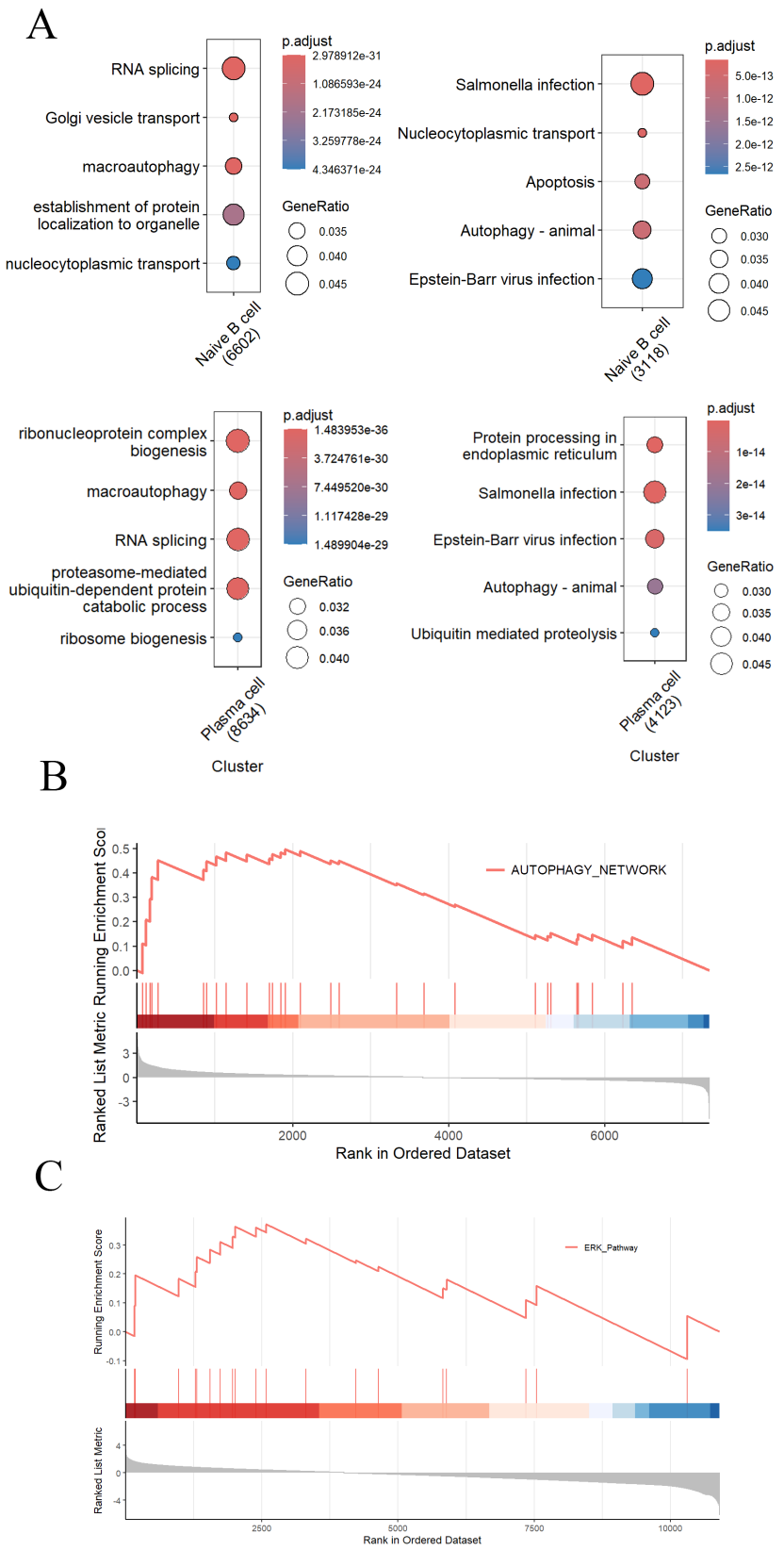

Fig. 1. Single-cell sequencing reveals alterations in autophagy and the ERK pathway. (A) Autophagy (macroautophagy) was altered in naïve B cells and plasma cells. (B) Expression of autophagy-related genes was upregulated in B cells. (C) The ERK pathway was altered in plasma cells. Abbreviations: ERK, extracellular signal-regulated kinase.

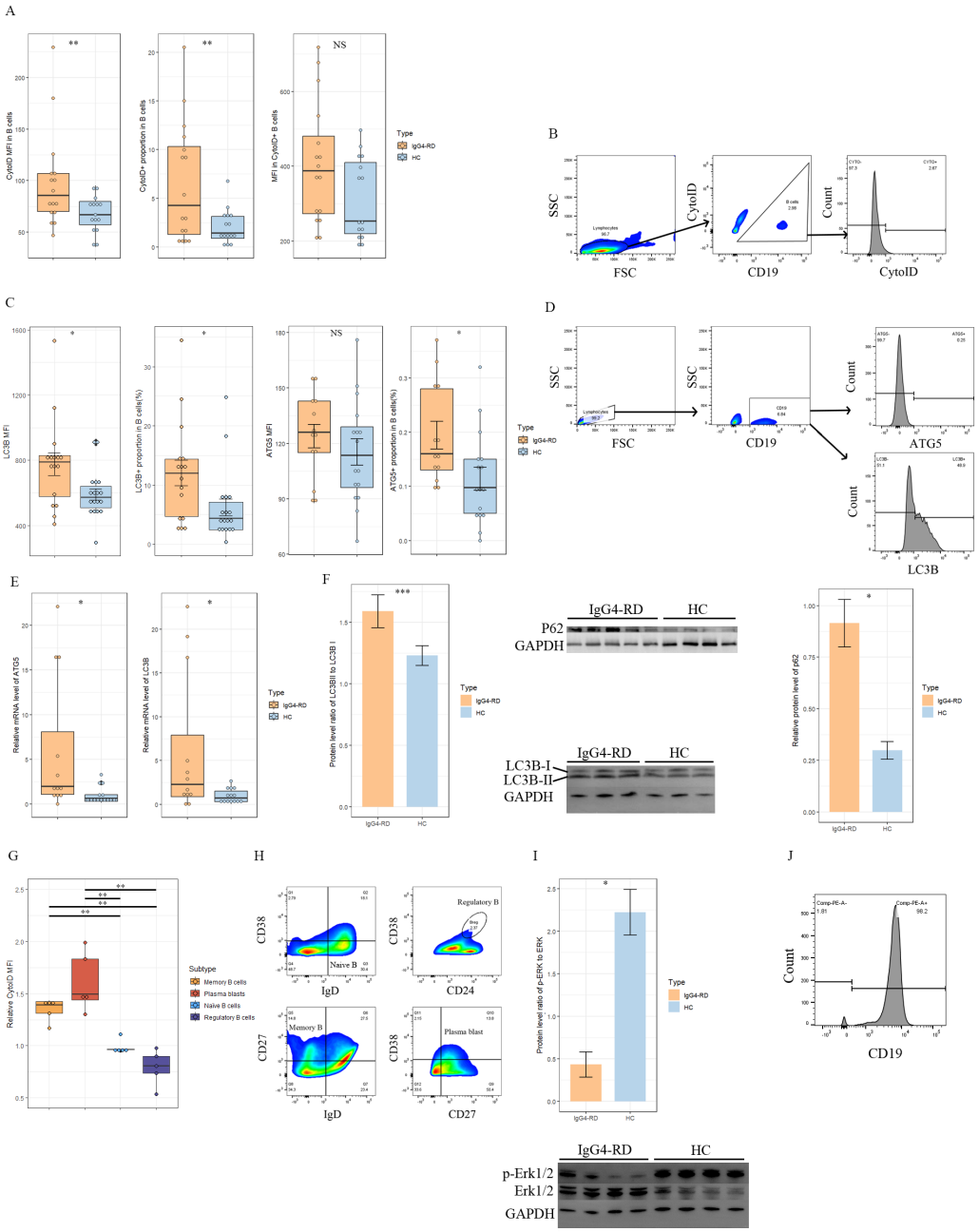

Fig. 2. Enhanced autophagy in B cells from IgG4-RD patients. (A) CytoID MFI and the proportion of CytoID$^+$ B cells were higher in IgG4-RD patients than in HCs ($p < 0.05$). CytoID MFI within CytoID$^+$ B cells was comparable between IgG4-RD and HCs ($p > 0.05$) (n = 16). (B) Flow cytometry plots showing the selection of CD19$^+$ B cells from PBMCs and detection of CytoID fluorescence. (C) LC3B MFI and LC3B$^+$ cell proportion were significantly higher in IgG4-RD ($p < 0.05$) (n = 16). (D) Flow cytometry plots showing CD19$^+$ B cell selection from PBMCs and detection of ATG5/LC3B fluorescence. (E) mRNA expression level of *LC3B* and *ATG5* were significantly higher in IgG4-RD (n = 12). (F) Protein level of P62 and the LC3B-II/LC3B-I ratio were elevated in IgG4-RD (n = 5, n = 12, respectively). (G) $Relative\ MFI = \frac{MFI\ of\ a\ B-cell\ subset}{MFI\ of\ total\ B\ cell}$. Plasma cells and memory B cells showed a relative CytoID MFI >1, whereas regulatory B cells and naïve B cells showed values <1 (n = 5). (H) Flow cytometry plot identifying naïve B cells (CD38$^-$, IgD$^+$), regulatory B cells (CD38^{hi}, CD24^{hi}), memory B cells (CD27$^+$, IgD$^-$), and plasmablasts (CD27$^+$, CD38$^+$) from CD19$^+$ B cells in PBMCs. (I) Phosphorylation levels of ERK1/2 were reduced in IgG4-RD (n = 4). (J) Purity verification for positive selection of B cells (NS $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: IgG4-RD, Immunoglobulin G4-related disease; CytoID, dye of CYTO-ID® Autophagy Detection Kit 2.0; MFI, mean fluorescence intensity; HCs, healthy controls; PBMCs, Peripheral blood mononuclear cells; LC3B, Microtubule-Associated Protein 1 Light Chain 3; ATG5, autophagy-related gene 5.

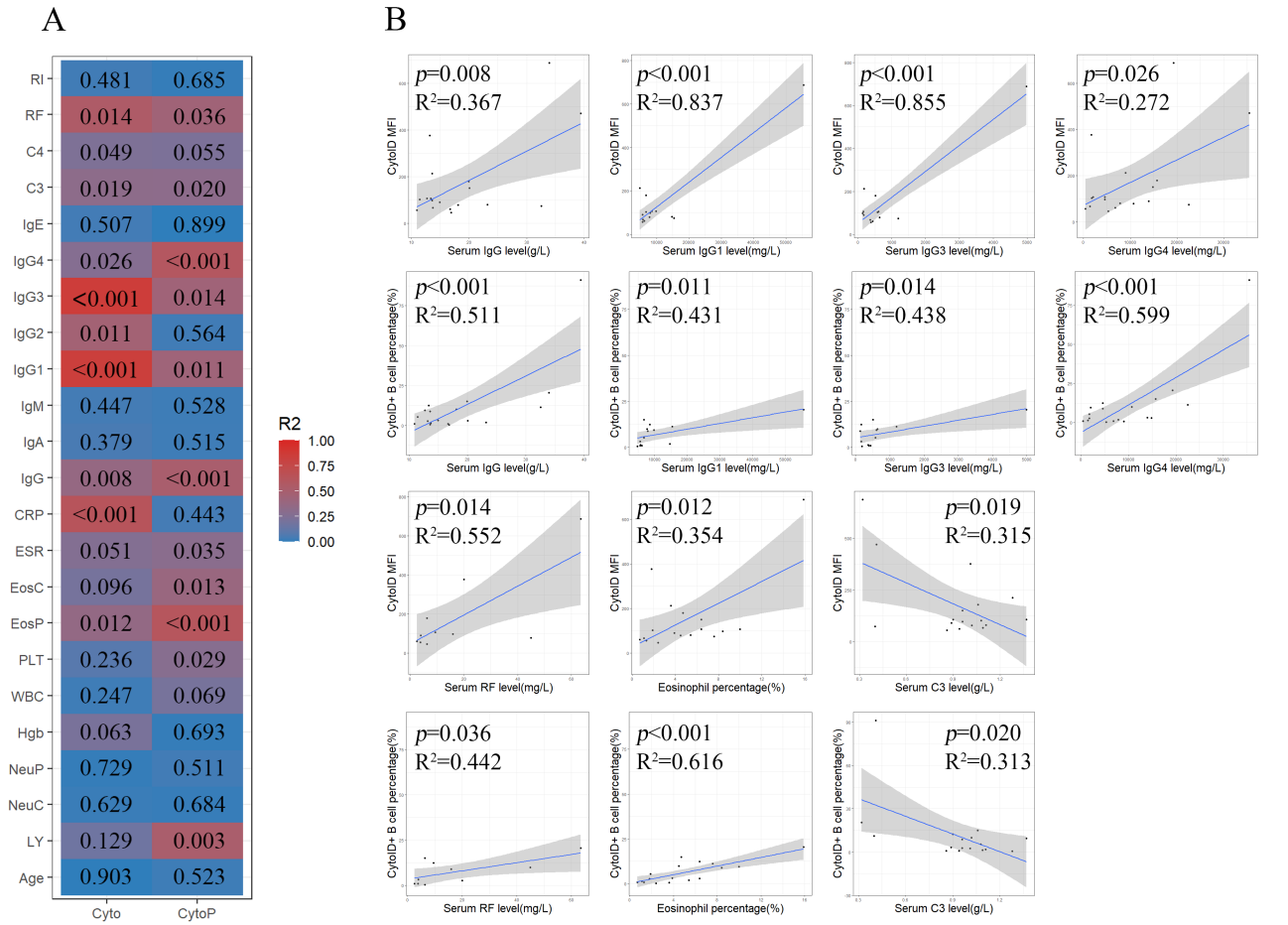

Fig. 3. Clinical parameter and B-cell autophagy levels (n = 18). (A) Correlations between clinical parameters and either CytoID (dye of CYTO-ID® Autophagy Detection Kit 2.0) MFI or the percentage of CytoID$^+$ B cells. *p*-values are indicated numerically within the heatmap, while R^2 values are represented by color intensity. (B) Correlations between serum IgG, IgG1, IgG3, IgG4, RF, C3 levels, or eosinophil percentage and either CytoID MFI or the percentage of CytoID$^+$ B cells. Abbreviations: LY, lymphocytes count; NeuC, neutrophil count; NeuP, neutrophil percentage among white blood cells; Hgb, hemoglobin level; WBC, white blood cell count; PLT, platelet count; EosP, eosinophil percentage among white blood cells; EosC, eosinophil count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; IgE, immunoglobulin E; C3, complement 3; C4, complement 4; RF, rheumatoid factor; RI, responder index; Cyto, CytoID MFI; CytoP, CytoID$^+$ B cell percentage.

difference was observed in the CytoID MFI of CytoID$^+$ B cells between groups ($p > 0.05$, Fig. 2A,B). Flow cytometry revealed elevated Microtubule-Associated Protein 1 Light Chain 3 (LC3B) MFI in IgG4-RD B cells ($p < 0.05$, Fig. 2C), along with an increased proportion of autophagy-related gene 5 (ATG5) /LC3B-high-expressing cells ($p < 0.05$, Fig. 2C,D). Consistently, ATG5 and LC3B mRNA expression levels were higher in IgG4-RD patients ($p < 0.05$, Fig. 2E). The ratio of LC3B type II to type I and the protein level of P62 were markedly elevated in IgG4-RD ($p < 0.001$ and $p < 0.05$, respectively, Fig. 2F).

Analysis of B cell subsets revealed that memory B cells and plasmablasts exhibited higher autophagy levels, whereas naïve B cells and regulatory B cells exhibited lower levels ($p < 0.01$, for each, Fig. 2G,H). Interestingly, ERK phosphorylation was markedly reduced in IgG4-RD compared to HCs ($p < 0.05$, Fig. 2I).

Moreover, several clinical parameters correlated with autophagy levels (Fig. 3A). Serum IgG, IgG1, IgG3, IgG4, and rheumatoid factor (RF) levels, as well as eosinophil percentage, were positively correlated with CytoID MFI or the proportion of CytoID$^+$ B cells, whereas serum complement 3 (C3) levels showed a negative correlation ($p < 0.05$, Fig. 3B).

Autophagy Inhibition Suppresses B-Cell Activity

Autophagy in B cells from IgG4-RD patients was effectively inhibited by 3-MA and BafA1 ($p < 0.05$, Fig. 4A). Following autophagy inhibition, OD_{450} values from the CCK-8 assay, representing metabolic activity, were signif-

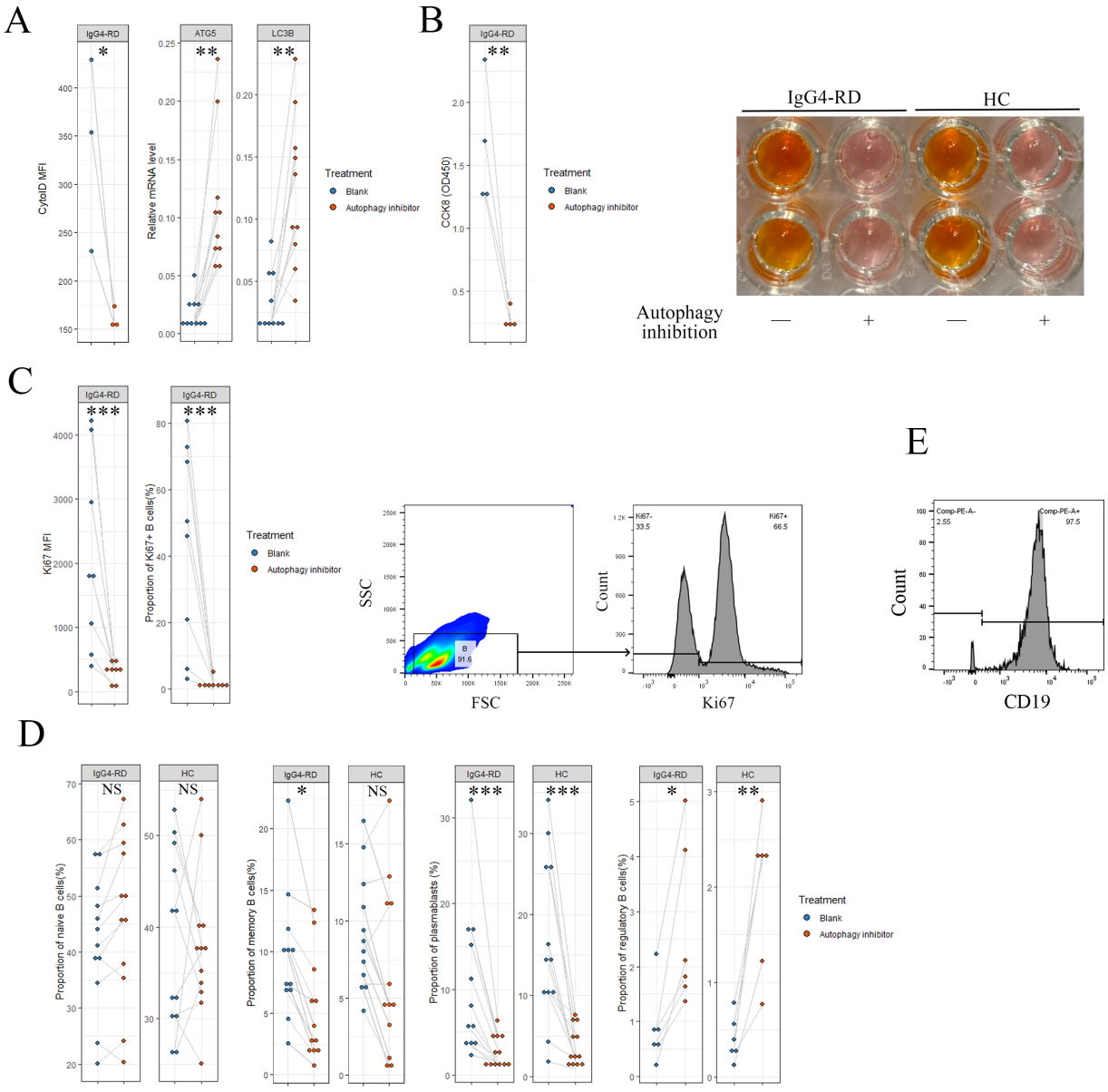

Fig. 4. Impact of autophagy inhibition on B-cell function. (A) Autophagy levels significantly decreased following autophagy inhibition treatment (n = 3, n = 10 respectively). (B) B-cell metabolic activity decreased after autophagy inhibition treatment (n = 4). (C) Proliferative capacity decreased after autophagy inhibition treatment (n = 8). Flow cytometry plots show gating for viable cells and detection of Ki-67 fluorescence. (D) The proportions of plasmablasts and regulatory B cells were significantly altered in both IgG4-RD and HC groups, whereas the proportion of memory B cells changed only in the IgG4-RD group after autophagy inhibition (n = 12). Flow cytometry plots are identical to those shown in Fig. 2H. (E) Purity verification for negative selection of B cells. NS $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

icantly reduced ($p < 0.01$, Fig. 4B). In parallel, Ki-67 expression was significantly decreased, both in MFI and in the proportion of Ki-67$^+$ B cells ($p < 0.001$ for each, Fig. 4C).

Comparative analysis of B cell subsets before and after autophagy inhibition revealed a significant reduction in memory B cells and plasmablasts in IgG4-RD ($p < 0.05$ and $p < 0.001$, respectively) and in plasmablasts in HCs ($p < 0.001$). Conversely, regulatory B cells were increased in both IgG4-RD and HCs ($p < 0.05$ and $p < 0.01$, respectively, Fig. 4D).

Cytokine profiling of B cell culture supernatants identified IL-6 as significantly decreased after autophagy in-

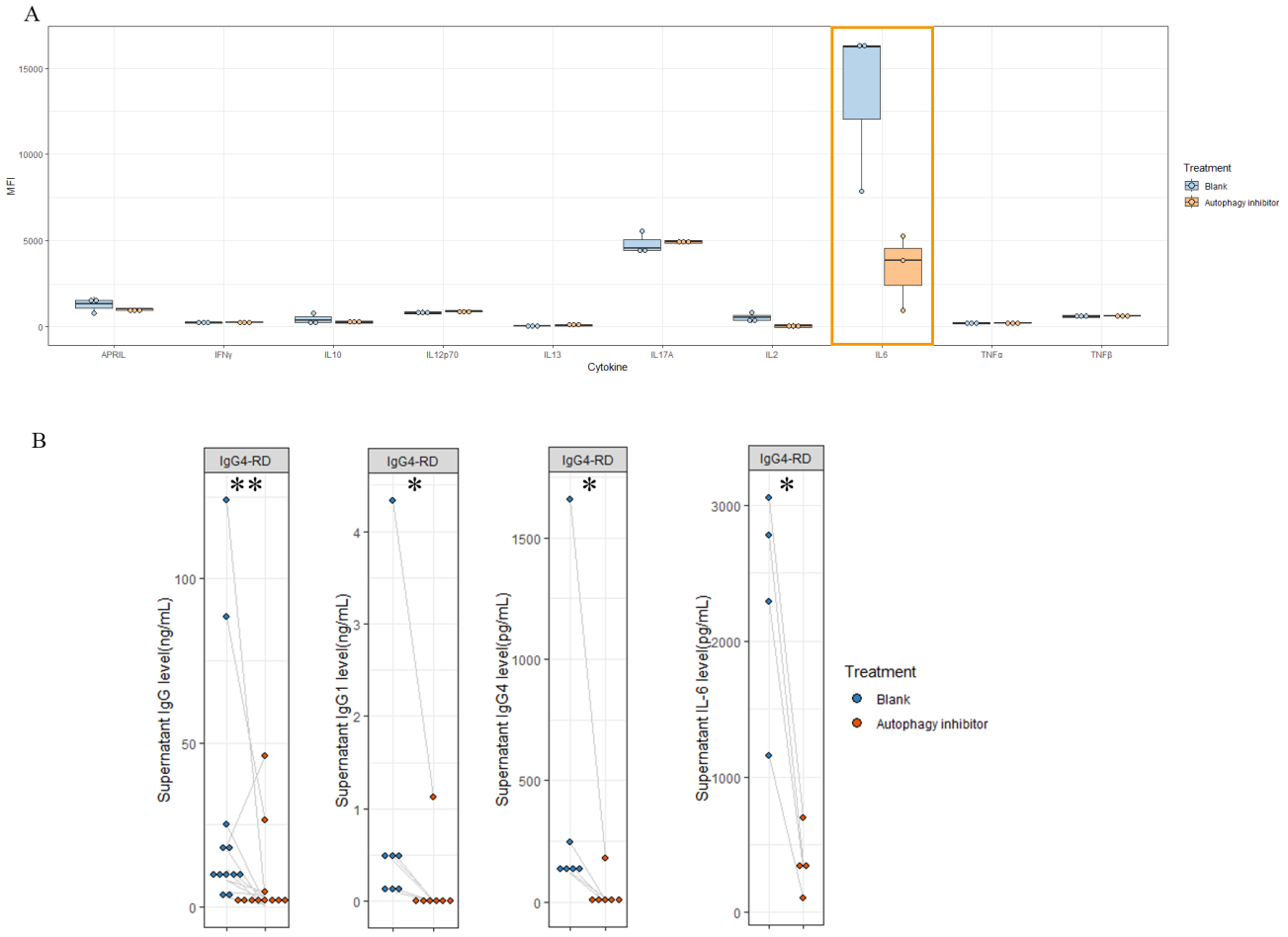

Fig. 5. Changes in serum cytokine and immunoglobulin levels following autophagy inhibition. (A) IL-6 levels decreased significantly after autophagy inhibition in both IgG4-RD and HC groups (n = 3). (B) ELISA confirmed significant reductions in serum IgG, IgG1, IgG4, and IL-6 levels following autophagy inhibition (n = 12). * $p < 0.05$, ** $p < 0.01$. Abbreviations: ELISA, Enzyme-linked immunoassay.

hibition, a finding confirmed by ELISA (Fig. 5A,B). In addition, antibody secretion was suppressed following autophagy inhibition, with IgG1 and IgG4 levels showing significant reduction ($p < 0.05$, Fig. 5B).

Discussion

To our knowledge, this is the first study to demonstrate enhanced autophagy in B cells of patients with IgG4-RD. Several proteins involved in the autophagy flux were upregulated at both transcriptional and translational levels. Autophagy intensity, reflected by CytoID MFI in B cells, was significantly increased in IgG4-RD. This elevation appeared to be driven by a higher proportion of B cells with elevated autophagy levels, as represented by the increased percentage of CytoID$^+$ B cells. These findings suggest a greater accumulation of autophagic vacuoles in B cells from patients with IgG4-RD.

Previous studies have shown that distinct autophagy-related proteins contribute to various clinical conditions [26]. In our study, autophagy intensity was positively correlated with peripheral eosinophil counts and serum levels of specific antibodies (IgG, IgG1, IgG4, and RF), showing a negative correlation with complement 3 (C3). This pattern indicates that B-cell autophagy intensity may serve as a surrogate marker of disease activity. Furthermore, other inflammatory and immune parameters such as ESR, CRP, and lymphocyte count, also correlated with CytoID MFI or the proportion of CytoID$^+$ B cells. Together, these findings support the hypothesis that intensified autophagy may contribute to the pathogenesis of IgG4-RD and is likely linked to its disease activity.

The ratio of LC3B-II to LC3B-I and the protein content of p62 were significantly elevated in patients, indicating impaired autophagic flux, particularly in the elongation and degradation phases. Similar alterations have been observed in motor neurons during aging [27]. For example, gamabufotalin (CS-6) exerts its anti-tumor effects in colorectal cancer by disrupting autophagy, leading to p62 ac-

cumulation and DNA damage [28]. Our findings provide the first evidence of defective autophagy in B cells of IgG4-RD. These cells, under sustained stress from excessive antibody production, may acquire features of cellular aging. Impairment in the degradation phase of autophagy could account for the observed accumulation of autophagic vacuoles.

Autophagy is known to play a pivotal role in plasma cell differentiation [13,22]. Experimental disruption of autophagy, either by genetic knockout of autophagy-related proteins or pharmacological inhibition using agents such as 3-methyladenine, bafilomycin A1, or chloroquine, has been shown to alter B-cell function and modulate immune responses [9,11–13,22]. In the present study, inhibition of autophagy led to a marked reduction in B-cell functions, including metabolism, proliferation, differentiation, and the secretion of antibodies and pro-inflammatory cytokines. These findings highlight the pivotal role of autophagy in sustaining B-cell activity and suggest its significant contribution to the pathogenesis of IgG4-RD.

However, the upstream mechanisms underlying abnormal autophagy activation remain unclear. In this study, compared to HCs, patients with IgG4-RD exhibited significantly reduced phosphorylation levels of ERK1/2, suggesting that downregulated ERK1/2 phosphorylation may contribute to abnormal B-cell autophagy in IgG4-RD. Interestingly, previous research has shown that IL-17-induced autophagy increases ERK1/2 phosphorylation, whereas inhibition of autophagy restores these enhanced levels [13]. In osteoarthritis, phosphorylation of ERK by FBXO21 inhibits cartilage autophagy [29], and in B-cell acute lymphoblastic leukemia, phosphorylated ERK1/2 was similarly found to suppress autophagy [30]. Although the role of ERK1/2 in autophagy remains controversial, accumulating evidence supports the hypothesis that ERK1/2 phosphorylation negatively regulates autophagy in B cells.

The precise causes of abnormal autophagy in IgG4-RD warrant further investigation. Advanced techniques, such as transmission electron microscopy, could be employed to better characterize autophagic flux. Additionally, future studies should explore other proteins involved in the autophagy pathway and experimentally manipulate ERK1/2 phosphorylation in HCs to mimic the IgG4-RD phosphorylation pattern, to determine the role of the ERK pathway in autophagy dysregulation. It will also be critical to identify upstream alterations in the ERK pathway that may drive the fundamental pathogenic mechanisms of IgG4-RD.

Several limitations should be acknowledged. The lack of age matching between the patient and healthy control groups may have influenced the observed differences in B-cell autophagy, as the HC was relatively younger. Furthermore, lifestyle factors were not considered during group matching, which may have introduced additional bias in the comparison of B-cell autophagy levels.

Conclusion

Patients with IgG4-RD exhibited defective autophagy in B cells, with increased autophagy levels correlating with multiple serological and hematological markers, as well as impaired autophagic flux. Among the examined signaling pathways, ERK1/2 phosphorylation was markedly reduced, suggesting potential interplay between autophagy and apoptosis. Inhibition of autophagy resulted in a generalized functional decline in B cells, alongside an anti-inflammatory phenotype. These findings highlight a potential link between autophagy and B cell activity, which may serve as a valuable biomarker for monitoring IgG4-RD disease status.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Author Contributions

Conceptualization, WZ and BS; methodology, YP, BS and WZ; software, BS and YN; validation, LP, YF, JZ and WZ; formal analysis, BS and WZ; investigation, BS; sample obtaining, YN, XH, JL, RS and NZ; data curation, BS, XH and RS; writing—original draft preparation, BS; visualization, BS; supervision, LP, YF, JZ and WZ; project administration, WZ; funding acquisition, WZ; writing—review and editing, JZ and WZ. All authors have been involved in revising it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This investigation received approval from the ethnic committee of Peking Union Medical College Hospital (No. S-442), with written informed consent obtained from all subjects. This study was conducted in adherence to the tenets of the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations.

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Conflict of Interest

The authors declare no conflict of interest. All people listed in the acknowledgement have agreed to use their names, with no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537200.163.

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