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ORIGINAL RESEARCH

Induction of interleukin 21 receptor expression via enhanced intracellular metabolism in B cells and its relevance to the disease activity in systemic lupus erythematosus

Masanobu Ueno ,[1] Shigeru Iwata ,[1,2] Kaoru Yamagata,[1] Yasuyuki Todoroki,[1,3] Koshiro Sonomoto ,[1,4] Atsushi Nagayasu,[1] Ippei Miyagawa,[1] Satoshi Kubo ,[1,3] Yusuke Miyazaki ,[1] Hiroko Miyata,[1] Ryuichiro Kanda ,[1] Takafumi Aritomi,[1] Shingo Nakayamada,[1] Yoshiya Tanaka [1]

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For numbered affiliations see end of article.

Correspondence to
Professor Yoshiya Tanaka;
tanaka@med.uoeh-u.ac.jp

ABSTRACT

Objective To elucidate the association between the changes in intracellular metabolism in the early stage of B cell activation and systemic lupus erythematosus (SLE) pathogenesis.

Methods $CD19^+$ or $CD19^+CD27^-$ (naïve) cells from the peripheral blood of healthy controls and lupus patients were cultured under different stimuli. The changes in intracellular metabolism and signalling pathways in these cells were evaluated.

Results Stimulation with CpG (Toll-like receptor 9 (TLR9) ligand) in vitro induced enhanced interleukin 21 (IL-21) receptor expression in $CD19^+CD27^-$ cells after 24 hours. The addition of IL-21 to the CpG stimulation enhanced the extracellular acidification rate, which indicates glycolysis, within 30 min. IL-21 receptor (IL-21R) expression induced by CpG stimulation was selectively inhibited by 2-deoxy-D-glucose (hexokinase 2 (HK2) inhibitor) and heptelidic acid (glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inhibitor). RNA immunoprecipitation with anti-GAPDH antibody revealed that CpG stimulation dissociated the binding between IL-21R messenger RNA (mRNA) and GAPDH under no stimulation. HK2 and GAPDH expression were higher in $CD19^+CD27^-$ cells of lupus patients than in those of healthy controls, and GAPDH expression was correlated with the plasmocyte count and disease activity score.

Conclusion IL-21R mRNA-GAPDH binding dissociation associated with rapid glycolytic enhancement by the TLR9 ligand in B cells may induce plasmocyte differentiation through IL-21 signals and be involved in exacerbating SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ B cells play a central role in the pathogenesis of systemic lupus erythematosus (SLE). Recent studies have shown the importance of immunometabolism as an intracellular and extracellular regulatory mechanism in the activation. While B cells produce copious amounts of autoantibodies, contributing to disease progression, this process requires producing a substantial amount of energy, proteins and nucleic acids. Elucidation of regulatory mechanisms in the activation and differentiation of B cells through immunometabolism may be a new method to elucidate the intracellular and extracellular events and SLE pathogenesis.

WHAT THIS STUDY ADDS

⇒ Detailed studies on the intracellular metabolic mechanism and its abnormalities in B cells from healthy individuals or patients with SLE are limited. In this study, interleukin 21 (IL-21) receptor messenger RNA-glyceraldehyde 3-phosphate dehydrogenase-binding dissociation associated with rapid glycolytic enhancement by the Toll-like receptor 9 ligand in B cells may induce plasmocyte differentiation through IL-21 signals and be involved in exacerbating SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The current study revealed the intracellular metabolic mechanism in the early stage of B cell activation, which triggers B cell activation and disease progression in patients with SLE. Enhanced glycolysis may be a new marker for the pathological condition exacerbating SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that commonly affects young women and damages various organs in the body, including the skin, joints, kidneys and nerves. While various immunocompetent cells are involved in SLE pathogenesis, B cells play a central role in the pathogenesis of SLE.[1–4] In B cells, diverse downstream

signal transduction pathways are induced mainly by B cell receptor (BCR) crosslinking, costimulators (such as soluble CD40 ligand (sCD40L)), Toll-like receptors (TLRs) and cytokines (such as interferon (IFN) α and interleukin 21 (IL-21)). Specifically, self-nucleic acids are known to activate TLR9, which induces B cell proliferation and differentiation into plasmocytes. It has been reported that TLR9 expression levels are correlated with Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), anti-double-stranded DNA antibodies and proteinuria, indicating that TLR9 is integrally involved in the pathogenesis of SLE[5–7]. These processes lead to the activation of self-reactive B cells, differentiation of plasmocytes, class switching and excessive production of autoantibodies. Autoantibodies bind to self-nucleic acids to form immune complexes, which accumulate in tissues, activate complements and cause tissue damage. Our research group has also reported the importance of B cells in SLE.[8–11]

Recent studies have shown the importance of immunometabolism as an intracellular and extracellular regulatory mechanism in the activation and differentiation of immune cells and that immunometabolism is involved in cytokine production and cell differentiation.[12–16] While B cells produce copious amounts of autoantibodies, contributing to disease progression, this process requires producing a substantial amount of energy, proteins and nucleic acids. Expression of mammalian target of rapamycin complex 1 (mTORC1) and enhancement of glycolysis have been confirmed in the B cells of a murine lupus model.**17–19** In fact, aberrant intracellular metabolism occurs in all types of immunocompetent cells in SLE.[20–23] Therefore, intracellular metabolism may be involved in complex processes of aberrant B cell activation and antibody production in SLE. Elucidation of regulatory mechanisms in the activation and differentiation of B cells through immunometabolism may be a new method to elucidate the intracellular and extracellular events, and SLE pathogenesis.

However, detailed studies on the intracellular metabolic mechanism and its abnormalities in B cells from healthy individuals or patients with SLE are limited. In recent years, we have elucidated the intracellular metabolic mechanism in the B cells of patients with SLE.[24] Glutaminolysis-mediated enhancement of mitochondrial function and methionine-induced enhancer of zeste homolog 2) expression committed plasmocyte differentiation in the B cells of patients with SLE and were integral to SLE pathogenesis.[25 26] However, the changes in intracellular metabolism in the early stage of B cell activation in patients with SLE and the association of intracellular metabolism with classical stimulus signalling pathways, including BCR, costimulators, cytokines and TLRs, remain unknown.

Hence, the current study aimed to investigate the changes in intracellular metabolism in the early stage of B cell activation and the association between these changes and SLE pathogenesis.

METHODS

Cell isolation and stimulation

Fresh, non-frozen B cells were used in all experiments to avoid changes in B cell death or metabolic function. Healthy control samples were obtained from volunteers who were recruited from the facility's staff and provided informed consent. SLE patient samples were obtained from consenting patients who were admitted to our department or attended outpatient clinics. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy adults and SLE patients using a lymphocyte separation medium (Cedarlane Corporation). These cells were then treated with magnetic beads to isolate different types of B cells: $CD19^+$ B cells (BioLegend), $CD19^+CD27^-$ naïve B cells (BioLegend) and $CD19^+CD27^+IgG^+$ class-switched (CS) memory B cells (VELITAS). The purity of $CD19^+$, $CD19^+CD27^-$ and $CD19^+CD27^+IgG^+$ B cells was greater than 95%. These cells were suspended (2.0×10^5, 200 µL/well, 96-well plate) in RPMI 1640, to which 10% normal foetal bovine serum (Sigma-Aldrich) and 100 U/mL each of penicillin and streptomycin (Thermo Fisher Scientific) were added. In some experiments, glucose-free RPMI 1640 media was used for cell culture. These cells were cultured for 1 day. BCR (anti-human IgG+IgM (H+L); Jackson ImmunoResearch Inc.), sCD40L (PeproTech), ODN 2006 (CpG, TLR9 agonist; InvivoGen) and loxoribine (Lox, TLR7 agonist; InvivoGen) were used for stimulation. The final concentrations were set at 1.0 µg/mL for BCR, 1.0 µg/mL for sCD40L, 0.5 µM for CpG and 1.0 mM for Lox. The cells were pretreated for 30 min before stimulation in the experiments using the following metabolic inhibitors: 2-deoxy-D-glucose (2-DG) (hexokinase (HK) 2 inhibitor; Wako), oligomycin (ATP synthase inhibitor; Abcam), rapamycin (mTORC1 inhibitor; Selleck), UK5099 (glucose flux blocker; Cayman Chemical), etomoxir (fatty acid flux blocker; Sigma-Aldrich), bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulphide (BPTES: glutaminolysis inhibitor; Sigma-Aldrich), itaconic acid (phosphofructokinase (PFK) inhibitor; Adipogen Life Sciences), heptelidic acid (glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitor; Adipogen Life Sciences), TEPP46 (pyruvate kinase M2 (PKM2) inhibitor; MedChemExpress), GSK2837808A (lactate dehydrogenase (LDH) inhibitor; Adipogen Life Sciences) and dichloroacetate (pyruvate dehydrogenase kinase (PDK) inhibitor; Sigma-Aldrich) were used as metabolic inhibitors.

Flow cytometry

$CD19^+$ cells were suspended in 100 µL of fluorescence-activated cell sorting (FACS) solution (0.5% human albumin and 0.1% NaN_3 in phosphate buffered saline) after washing, followed by staining with fluorochrome conjugated anti-human antibodies (listed in online supplemental table 1) for 30 min. For exclusive analysis of live populations, these cells were also stained with a Fixable Viability Dye (e-Biosciences) and finally analysed using the FACS Lylic (BD Biosciences)/FlowJo v10

software (TOMY Digital Biology, Tokyo, Japan). For intracellular staining, cells were first fixed and permeabilised with a Transcription Factor Buffer Set (BD Biosciences) and washed with fluorescence-assisted cell sorting solution. The gating strategy is shown in online supplemental figure S1A. The expression of various cytokines is presented as the difference in MFI (ΔMFI) compared with the isotype control (online supplemental figure S1B).

Extracellular flux analysis

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of $CD19^+CD27^-$ B cells (naïve B cells) obtained from healthy controls were measured using an XF96 Extracellular Flux analyser (Seahorse Bioscience, North Billerica, MA, USA). $CD19^+CD27^-$ B cells (naïve B cells) were not cryopreserved but were isolated immediately after blood collection for metabolic function assessment. Experiments were repeated using B cells from four different healthy individuals. $CD19^+CD27^-$ B cells (naïve B cells) were suspended in XF media (Agilent Technologies, Santa Clara, CA, USA) supplemented with 1 mM sodium pyruvate (Agilent Technologies), 10 mM glucose (Agilent Technologies) and 2 mM L-glutamine (Thermo Fisher Scientific) and placed in XF96 cell culture microplates (2×10^5 cells per well) coated with Cell-Tak (BD Biosciences). The OCR and ECAR were measured using the XF media under basal conditions for 30 min. Subsequently, 1.0 µg/mL for BCR, 1.0 µg/mL for sCD40L, 0.5 µM for CpG and 1.0 mM for Lox were injected, and metabolic function was measured for 2 hours. After 2 hours, the OCR and ECAR were measured in response to oligomycin (2 µM), carbonyl cyanide-*p*-trifluoromethoxy-phenylhydrazone (FCCP, 2 µM) and rotenone (0.5 µM)/antimycin A (Rot/AA) (0.5 µM), and we checked the assay actually worked.

Lactate assay

$CD19^+CD27^-$ B cells (naïve B cells) were cultured for 2 hours in 96-well plates. The medium and supernatant lactate contents were measured spectrophotometrically using a Lactate Assay Kit II according to instructions from the manufacturer (BioVision).

Real-time quantitative PCR

Total RNA was extracted using the RNeasy Mini Kit and used to synthesise complementary DNA (cDNA). After that, quantitative real-time PCR (RT-PCR) was performed using the Step One Plus instrument (Applied Biosystems, Waltham, MA, USA) in triplicate in 96-well plates. The TaqMan and SYBR Green target lists are shown in online supplemental table S2. The expression of each mRNA was normalised to that of the endogenous control 18S ribosomal RNA (rRNA), and gene expression levels were presented as relative quantification.

RNA immunoprecipitation

The cells (1 condition, 1×10^6) were fixed with formaldehyde, washed with cold phosphate buffered saline, suspended in 500 µL of nuclei isolation buffer (1.28 M sucrose, 40 mM Tris-HCl pH=7.5, 20 mM $MgCl_2$, 4% Triton X-100) and incubated in ice for 15 min. After spin-down, the pellets were suspended in 500 µL of lysis buffer (50 mM KCl, 25 mM tris-HCl, 5 mM ethylenediaminetetraacetic acid, 0.5 mM dithiothreitol, 0.5% Nonidet P40) and RNA was sonicated to achieve a length range of 200–1000 bases. Subsequently, 50 µL of the resultant sample was stored at –80°C as input, and the remainder was added to magnetic beads prebound to anti-GAPDH antibody or non-specific IgG and incubated overnight while being rotated. After washing, the incubated sample was suspended in 200 µL of elution buffer (1% sodium dodecyl sulphate, 0.1 M $NaHCO_3$, proteinase K and ribonuclease (RNase inhibitor) 0.5 µL) and incubated at 65°C for 30 min to elute RNA (the input RNA was also eluted in the same manner). RNA was separated with phenol-chloroform-isoamyl alcohol, precipitated with ethanol and re-suspended in RNase free water. cDNA was prepared as described above, and quantitative RT-PCR was performed. The adenylate/uridylate (AU)-rich element (ARE) of IL-21 receptor (IL-21R) mRNA was identified by searching the base sequence data in the National Library of Medicine. The absence of ARE regions in IFN-γ receptor 1 (IFNGR1) mRNA was confirmed similarly.

Patients

The participants were patients with SLE, which was classified according to the 2019 European League Against Rheumatology/American College of Rheumatology classification criteria for SLE and age-matched healthy donors. The patient's clinical features are listed in online supplemental table S3. $CD19^+CD27^-$ B cells were isolated from PBMCs, and the gene expression in the B cells was evaluated using quantitative RT-PCR from 30 patients with SLE and 10 healthy donors. Patients with SLE were divided into two groups based on the British Isles Lupus Assessment Group (BILAG) index, which is an SLE activity index: the high-disease activity group comprised patients with SLE with BILAG A for one domain or BILAG B for two organ domains, and the low-disease activity group comprised the remaining patients. Ethical approval was obtained from the Japan Ethics Committee of the University of Occupational and Environmental Health, Japan (approval number #UOEHCRB19-046, #UOEHCRB21-069). The study complied with the Declaration of Helsinki. Informed consent was obtained from all patients enrolled in the LOOPS Registry and healthy controls.

Statistical analysis

Data were expressed as the median (IQR, interquartile range) or numbers (%). Statistically significant differences between the two groups were examined using Pearson's test, Student's t-test and Wilcoxon's rank sum test. Differences among three or more groups were examined using the analysis of variance (ANOVA), Tukey–Kramer

test or Dunn's test. Pearson correlation coefficients were calculated to test the association between two variables of interest. Each test was performed, and statistical significance was set at $p<0.05$. Statistical analyses were conducted using JMP Pro V.15 (SAS Institute Inc., Cary, NC) and GraphPad Prism 9 (GraphPad Software, San Diego, CA).

RESULTS

CpG affects interleukin 21 (IL-21) receptor expression, B cell differentiation and glycolysis in CD19$^+$ cells

TLR7 and TLR9 expression were confirmed in B cells (online supplemental figure S2). Initial investigations were aimed at determining which stimuli induced the expression of these receptors in CD19$^+$ B cells (Pan B cells). CD19$^+$ B cells (Pan B cells) were isolated from the peripheral blood of healthy controls, stimulated with BCR, sCD40L, TLR ligand Lox (TLR7 ligand) and CpG (TLR9 ligand) and cultured for 24 hours. After that, flow cytometry was performed to evaluate the cell activation marker and cytokine receptor expression. The expression of CD86, a marker of cell activation, was upregulated in response to all the stimuli (online supplemental figure S3). Among the four types of stimuli, only CpG significantly increased IL-2 receptor (IL-2R), IL-21R and IFNGR1 expression. However, IL-4R, IL-7R, IL-9R, IL-15R and IFN-α receptor 2 (IFNAR2) expressions were not increased by any stimuli (figure 1A).

We then evaluated which B cell subsets expressed IL-2R, IL-21R and IFNGR1. B cells were divided into CD19$^+$CD27$^-$ B cells (naïve B cells) and CD19$^+$CD27$^+$IgG$^+$ B cells (CS memory B cells) to compare IL-2R, IFNGR1 and IL-21R expression, which were increased by CpG stimulation. Although the expression of all receptors was increased in both naïve B cells and CS memory B cells, no difference was observed in IL-2R expression between naïve B and CS memory B cells, whereas IL-21R and IFNGR1 expression was significantly higher in naïve B cells than in CS memory B cells (figure 1B). Subsequently, CD19$^+$CD27$^-$ B cells (naïve B cells) were cultured with CpG or no stimulation for 24 hours and stimulated with IL-21. In CD19$^+$CD27$^-$ B cells (naïve B cells) cultured for 24 hours with CpG stimulation, IL-21 stimulation led to a concentration-dependent increase in pSTAT3 (figure 1C).

Next, CD19$^+$CD27$^-$ B cells (naïve B cells) were stimulated with various stimuli (Lox, BCR, sCD40L, CpG), and changes in the OCR, which indicates the degree of oxidative phosphorylation, and the ECAR, which indicates the degree of glycolysis, were evaluated. The assay used in this study was functioning correctly (online supplemental figure S4A), as previously reported.[27] The OCR was not changed by any stimuli (figure 2A–C, online supplemental figure S4B). ECAR increased within 30 min after stimulation with BCR, sCD40L and CpG. ECAR increased significantly only after CpG stimulation compared with no stimulation. However, ECAR was not increased by stimulation with Lox (figure 2D–F, online supplemental file 1). After culturing CD19$^+$CD27$^-$ B cells (naïve B cells) for 2 hours with CpG stimulation, which most strongly enhanced glycolysis, there was a significant increase in lactate levels in the supernatant (figure 2G). When CD19$^+$CD27$^-$ B cells (naïve B cells) were cultured with CpG stimulation for 24 hours, the expression of mRNA for glucose transporter 1 (GLUT1) and glycolytic enzymes (HK2, PFK, GAPDH, PKM2 and LDH) was significantly higher than that with no stimulus exposure (figure 2H, online supplemental figure S4D). CpG stimulation led to an increase in IL-21R expression and enhanced glycolysis in CD19$^+$CD27$^-$ B cells (naïve B cells). We then investigated the mechanism of IL-21R expression in these cells, with a focus on immunometabolism.

Glycolytic enzymes, especially glyceraldehyde-3-phosphate dehydrogenase (GAPDH), regulate interleukin 21 receptor (IL-21R) expression in CD19$^+$ cells

The changes in receptor expression were examined using the inhibitors of various metabolic pathways to evaluate the presence or absence of a direct association of each metabolic pathway with IL-21R expression. The following inhibitors of metabolic pathways were used: glycolytic inhibitor, 2-DG; oxidative phosphorylation inhibitor, oligomycin; mTOR inhibitor, rapamycin; UK5099, which inhibits the transition from the glycolysis to the tricarboxylic acid (TCA) cycle; β-oxidation inhibitor, etomoxir; and glutaminolysis inhibitor, BPTES (figure 3A). ECAR was decreased by 2-DG, but not by oligomycin, rapamycin, UK5099, etomoxir and BPTES (figure 3B, online supplemental figure S5A). Cell viability did not decrease at the highest concentration of any of the inhibitors mentioned above (data not shown). IL-21R expression was significantly inhibited by 2-DG (a glycolytic inhibitor) in a concentration-dependent manner, but was not altered by the other five inhibitors that did not decrease the ECAR (figure 3C). IFNGR1 expression was not decreased by any of the metabolic inhibitors (online supplemental figure S6). When CD19$^+$CD27$^-$ B cells (naïve B cells) were stimulated with CpG for 24 hours using either regular media (glucose concentration 2.0 g/L) or glucose-free media, IL-21R expression was significantly lower in the glucose-free media cultures (figure 3D). We then investigated whether IL-21R expression was affected by glycolytic enzyme inhibitors other than HK2, which is a key enzyme at the entry point of glycolysis.

IL-21R expression was examined using glycolytic enzyme inhibitors other than 2-DG, such as itaconic acid (PFK inhibitor), heptelidic acid (GAPDH inhibitor), TEPP46 (PKM2 inhibitor), GSK28377808A (LDH inhibitor) and dichloroacetate (PDK inhibitor) (figure 4A). All glycolytic enzyme inhibitors lowered the ECAR compared with CpG stimulation (figure 4B, online supplemental figure S5B). No significant difference was observed in cell viability at the highest concentration of each inhibitor (data not shown). As observed with 2-DG, IL-21R expression was significantly decreased only by heptelidic

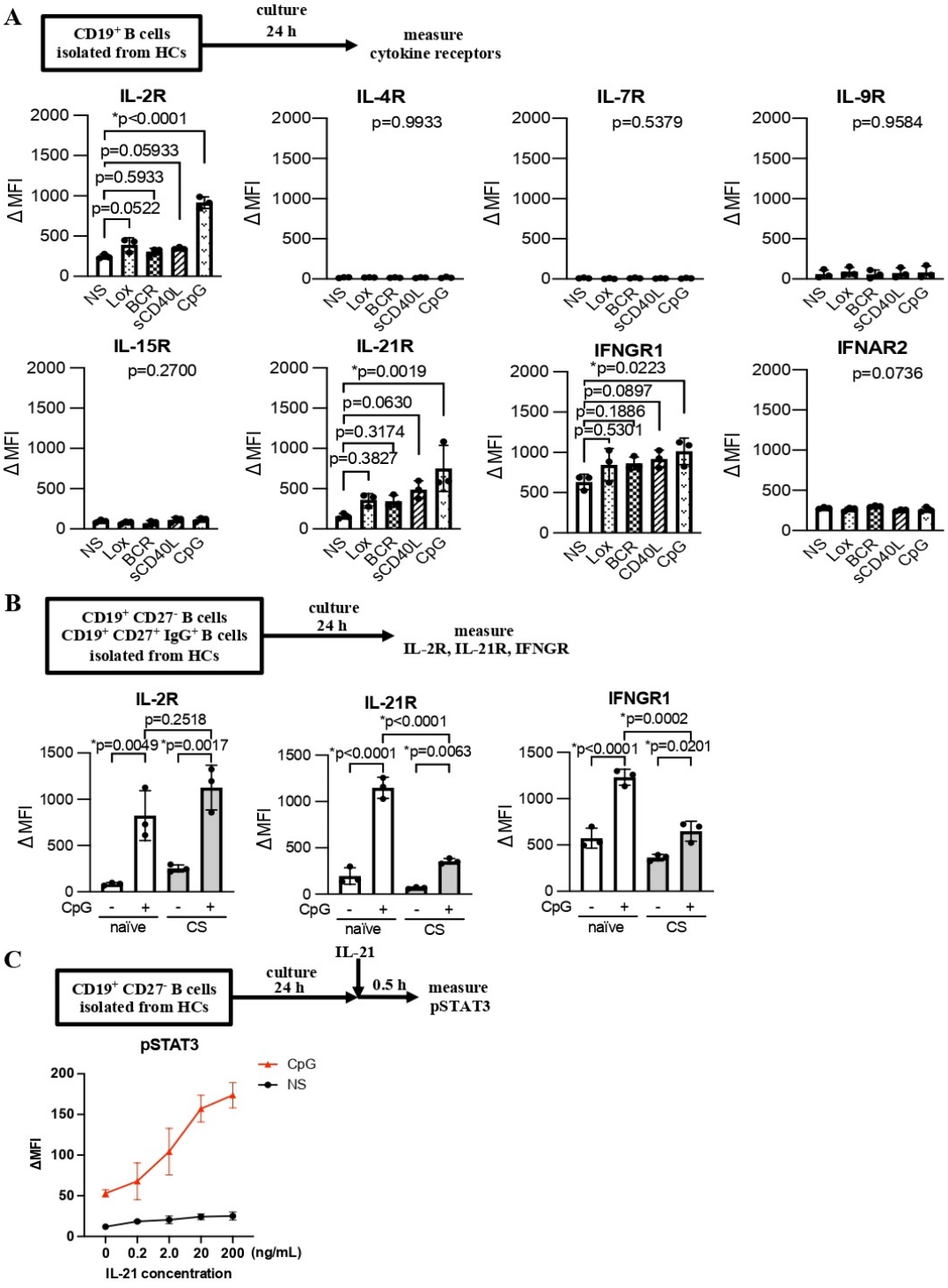

Figure 1 The stimulation by CpG induces IL-21R. CD19$^+$ B cells (Pan B cells), CD19$^+$CD27$^-$ B cells (naïve B cells) and CD19$^+$CD27$^+$IgG$^+$ B cells (class-switched (CS) memory B cells) were cultured with Lox 1.0 mM, BCR 1.0 µg/mL, sCD40L 1.0 µg/mL and CpG 0.5 µM for 24 hours. (A) Expression level of IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, IL-21R, IFNGR1 and IFNAR2 in cultured CD19$^+$ B cells (Pan B cells) (n=3). (B) Expression level of IL-2R, IL-21R and IFNGR1 in cultured CD19$^+$CD27$^-$ B cells (naïve B cells) and CD19$^+$CD27$^+$IgG$^+$ B cells (CS memory B cells) (n=3). (C) After the CD19$^+$CD27$^-$ B cells (naïve B cells) were cultured under CpG stimulation for 24 hours, they were separately stimulated with IL-21 (0 ng/mL, 0.2 ng/mL, 2.0 ng/mL, 20 ng/mL, 200 ng/mL) and cultured for 0.5 hours. Then, pSTAT3 was measured using flow cytometry (n=4). The bars indicate the mean±SD from three or four independent experiments using CD19$^+$ B cells (Pan B cells), CD19$^+$CD27$^-$ B cells (naïve B cells) and CD19$^+$CD27$^+$IgG$^+$ B cells (CS memory B cells) from healthy controls. ΔMFI indicates the difference from the isotype control. *P* values were determined using the analysis of variance (A), Tukey–Kramer (A) or Student's t-test after false discovery rate correction (B). $^*p<0.05$. HCs, healthy controls; NS, not stimulated; BCR, B cell receptor; IFN, interferon; IFNAR2, IFN-α receptor 2; IFNGR1, IFN-γ receptor 1; IL, interleukin; Lox, loxoribine; pSTAT3, phosphorylated signal transducer and activator of transcription 3; sCD40L, soluble CD40 ligand.

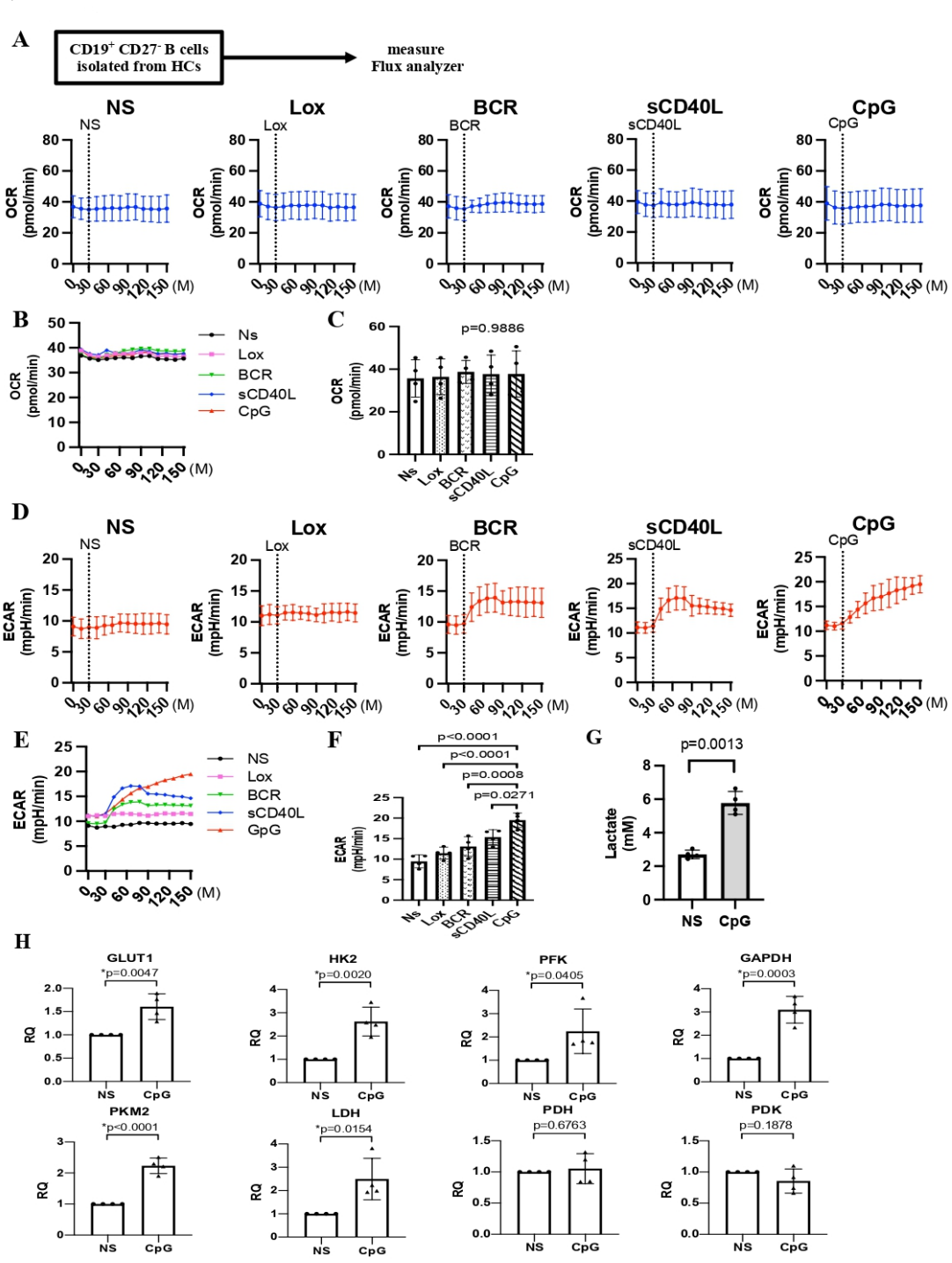

Figure 2 ECAR rapidly increased BCR, sCD40L and TLR9 (CpG) stimuli in CD19^{+}CD27^{-} B cells (naïve B cells) (A)-(F) CD19^{+}CD27^{-} B cells (naïve B cells) from healthy controls were stimulated with Lox 1.0 mM, BCR 1.0 µg/mL, sCD40L 1.0 µg/mL and CpG 0.5 µM. Metabolic function was evaluated with the flux analyser (OCR and ECAR) (n=4). (G) After CD19^{+}CD27^{-} B cells (naïve B cells) from healthy controls were stimulated with CpG 0.5 µM for 24 hours, lactic acid in supernatant was measured (n=4). (H) Gene expression in CD19^{+}CD27^{-} B cells (naïve B cells) after stimulation with CpG for 24 hours, as determined by the RT-PCR (n=4). (A)**(**B) Changes in the OCR. (C) Comparison of the OCR at 150 min after each stimulation. (D)**(**E) Changes in the ECAR. (F) Comparison of the ECAR at 150 min after each stimulation. (G) Concentration of lactic acid in supernatant. (H) Gene expression of GLUT1, HK2, PFK, GAPDH, PKM2, LDH, PDH and PDK. (A)–(H) The bars indicate the mean±SD from four independent experiments using CD19^{+}CD27^{-} B cells (naïve B cells) from healthy controls. *P* values were determined using the analysis of variance (C), Tukey–Kramer (F) or Student's t-test (G,H). p*<0.05. HCs, hearty controls; NS, not stimulated; BCR, B cell receptor; ECAR, extracellular acidification rate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT1, glucose transporter 1; HK2, hexokinase2; LDH, lactate dehydrogenase; Lox, loxoribine; OCR, oxygen consumption rate; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PKM2, pyruvate kinase M2; RT-PCR, real-time PCR; RQ, relative quantification; sCD40L, soluble CD40 ligand.

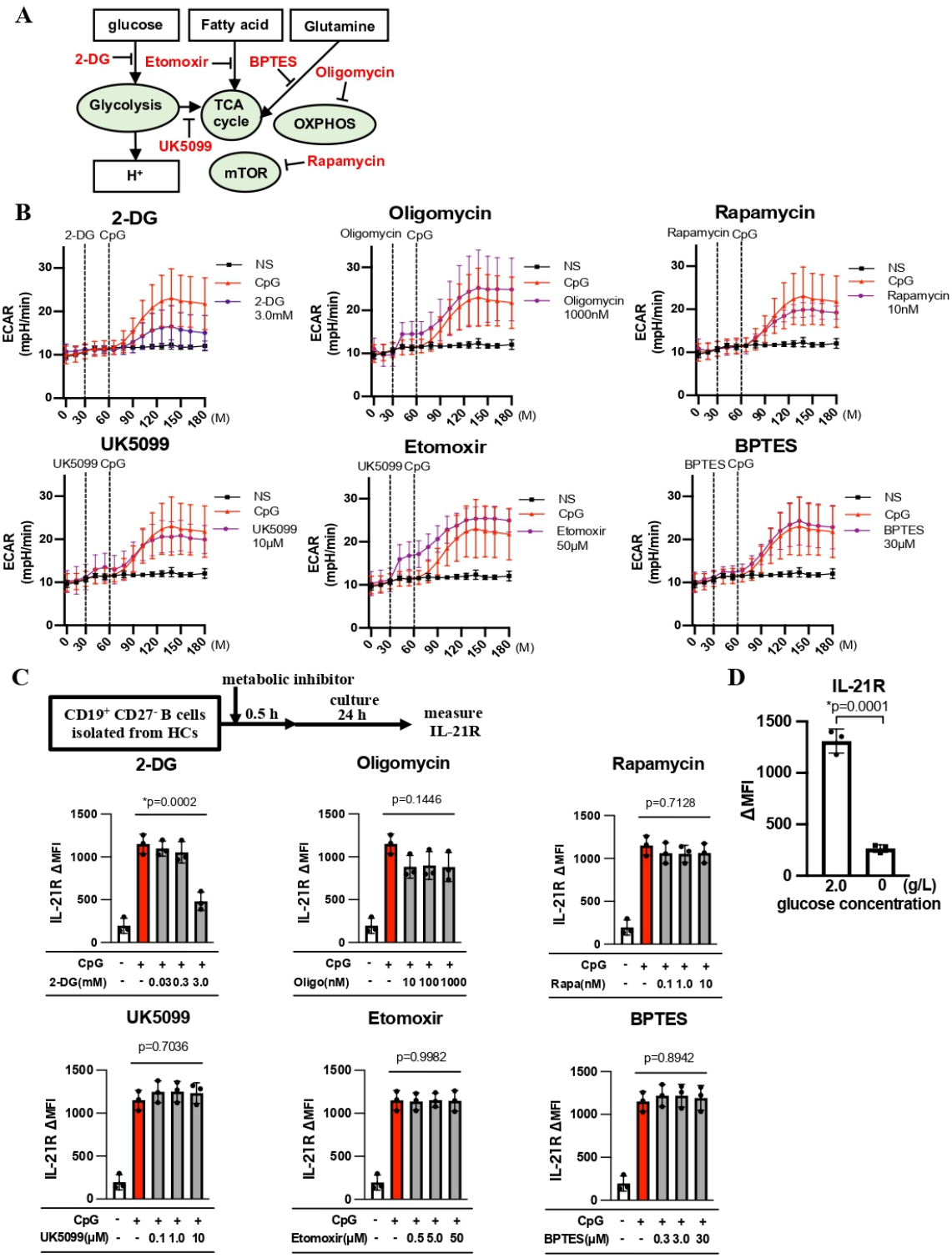

Figure 3 The alteration of IL-21R expression by metabolic inhibitors in naïve B cells After $CD19^+CD27^-$ B cells (naïve B cells) were pretreated with each metabolic inhibitor for 30 min; they were cultured with CpG stimulation for 24 hours. The expression level of IL-21R was measured using flow cytometry. (A) Overview of metabolic inhibitors. (B) Changes in the ECAR in $CD19^+CD27^-$ B cells (naïve B cells) after pretreatment with each metabolic inhibitor (n=3). (C) IL-21R expression in $CD19^+CD27^-$ B cells (naïve B cells) (n=3). (D) $CD19^+CD27^-$ B cells (naïve B cells) were cultured with CpG stimulation in glucose 2.0 g/L medium or glucose-free medium for 24 hours. IL-21R expression in $CD19^+CD27^-$ B cells (naïve B cells) (n=3). (A)–(C) The bars indicate the mean±SD from three independent experiments using $CD19^+CD27^-$ B cells (naïve B cells) from healthy controls. ΔMFI indicates the difference from the isotype control. *P* values were derived using the analysis of variance (C) or Student's t-test (D). $^*p<0.05$, $^{**}p<0.01$. HCs, healthy controls; NS, not stimulated; OXPHOS, oxidative phosphorylation; 2-DG, 2-deoxy-D-glucose; BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulphide; ECAR, extracellular acidification rate; IL-21R, interleukin 21 receptor; mTOR, mammalian target of rapamycin; TCA, tricarboxylic acid.

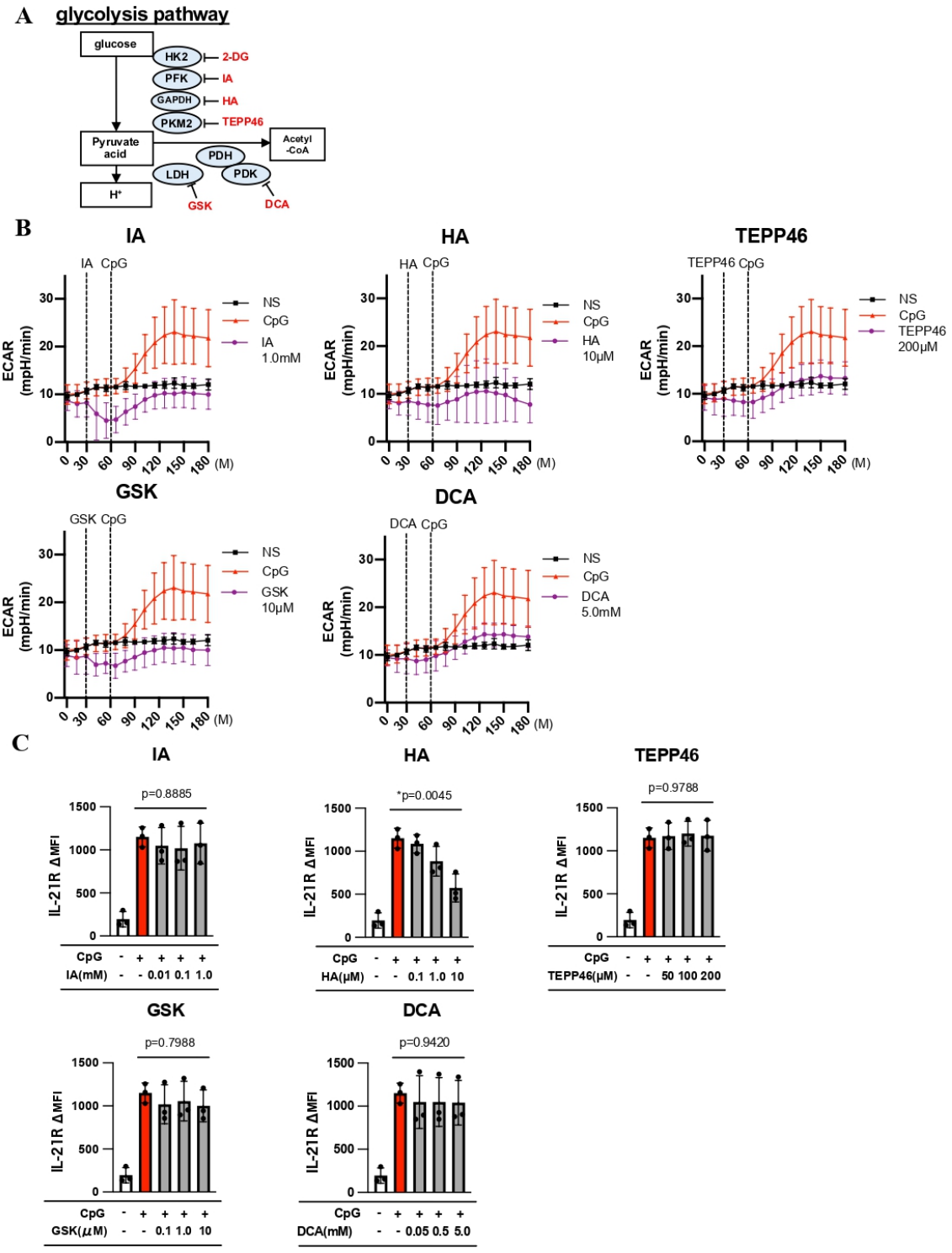

Figure 4 The alteration of IL-21R expression by glycolytic enzyme inhibitors in naïve B cells. First, $CD19^{+}CD27^{-}$ B cells (naïve B cells) from healthy controls were pretreated with each glycolytic enzyme inhibitor for 30 min, followed by culture with CpG stimulation for 24 hours. The expression level of IL-21R was measured using flow cytometry. (A) Overview of glycolytic enzyme inhibitors. (B) Changes in the ECAR in $CD19^{+}CD27^{-}$ B cells (naïve B cells) after pretreatment with each glycolytic enzyme inhibitor (n=3). (C) IL-21R expression in $CD19^{+}CD27^{-}$ B cells (naïve B cells) (n=3). (A)–(C) The bars indicate the mean±SD derived from three independent experiments using $CD19^{+}CD27^{-}$ B cells (naïve B cells) from healthy controls. ΔMFI indicates the difference from the isotype control. *P* values were derived using the analysis of variance test (C). $^{*}p<0.05$, $^{**}p<0.01$. NS, not stimulated; ECAR, extracellular acidification rate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IA, itaconic acid; HA, heptelidic acid; GSK, GSK2837808A; DCA, dichloroacetate; HK2, hexokinase 2; IL-21R, interleukin 21 receptor; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase; PFK, phosphofructokinase; PKM2, pyruvate kinase M2.

acid in a concentration-dependent manner, whereas no such decrease was observed for the other four glycolytic enzyme inhibitors (figure 4C).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibits protein translation by binding to interleukin 21 receptor (IL-21R) messenger RNA (mRNA)

Next, we investigated the mechanisms by which GAPDH regulates IL-21R expression. First, IL-21R mRNA expression was most enhanced 3 hours after CpG stimulation (figure 5A). IL-21R mRNA expression was not changed by the following metabolic pathway inhibitors, including glycolytic inhibitors, at the highest concentration: 2-DG, oligomycin, rapamycin, UK5099, etomoxir and BPTES (figure 5B). IL-21R protein expression decreased with 2-DG treatment, but IL-21R gene expression did not. This suggested that glycolysis is involved in posttranslational modification of IL-21R expression. GAPDH is known to inhibit protein translation by binding to AREs in mRNA.[28–31] We hypothesised that when glycolysis is enhanced by CpG stimulation, GAPDH bound to mRNA acts as a glycolytic enzyme and the binding with mRNA is broken, leading to increased protein translation (online supplemental figure S7). IL-21R mRNA contains AREs. To verify the binding of GAPDH to AREs, RNA immunoprecipitation (RIP) was performed with an anti-GAPDH antibody. IL-21R mRNA expression was evaluated by PCR using PCR probes without ARE (primers 1 and 3) and PCR probes containing ARE (primer 2). RIP with the anti-GAPDH antibody showed that the IL-21R mRNA expression evaluated with the ARE-containing PCR probe was significantly higher than that with the ARE-free PCR probes (figure 5C). This indicated that GAPDH is bound to the ARE regions of IL-21R mRNA. RIP with anti-GAPDH antibody showed that the expression of IFNGR1 mRNA was significantly lower than that of IL-21R mRNA (figure 5D). Next, RIP-PCR was performed with naïve B cells under the following conditions: no stimulation, CpG stimulation and CpG stimulation in combination with 2-DG or heptelidic acid. After CpG stimulation, that is, an enhanced state of glycolysis, the expression level of IL-21R mRNA was significantly lower compared with that after no stimulation and application of 2-DG and HA, that is, a state of unenhanced glycolysis (figure 5E).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression is increased and positively correlated with the absolute plasmocyte count and disease activity in naïve B cells from patients with systemic lupus erythematosus (SLE)

Finally, we evaluated the associations with glycolytic enzyme expression and clinical features in the $CD19^{+}CD27^{-}$ B cells of patients with SLE. The patient characteristics are presented in online supplemental tables S3, S4 and S5 and online supplemental figure S8. RT-PCR evaluation of the levels of IL-21R, GLUT1 and glycolytic enzymes in $CD19^{+}CD27^{-}$ B cells from healthy controls and patients with SLE revealed that the levels of IL-21R, GLUT1, HK2 and GAPDH were elevated in patients with SLE with high disease activity (figure 6A and B). The correlations of GLUT1 and HK2 with the clinical features are shown in online supplemental figure S9A,B. Furthermore, GAPDH expression was positively correlated with the SLEDAI 2000 (SLEDAI-2K) and absolute plasmocyte count and negatively correlated with 50% haemolytic complement activity (figure 6C, online supplemental figure S9C).

DISCUSSION

In recent years, the importance of immunometabolism as an intracellular and extracellular regulatory mechanism in the activation and differentiation of immune cells has garnered substantial attention. In the current study, we investigated the changes in intracellular metabolism in the early stage of human B cell activation and the association between these changes and SLE pathogenesis.

Stimulation and consequent activation of B cells lead to the enhancement of glucose uptake and induction of aerobic glycolysis.[32–34] Aerobic glycolysis not only rapidly produces energy but also induces the synthesis of intermediates essential for cell proliferation, such as fatty acids and nucleic acids, through the pentose phosphate pathway and plays an important role in mitochondrial maturation.[35–38] It has been reported that in B cells, various stimuli can enhance glycolysis, which is a crucial factor for B cell proliferation, differentiation and antibody production.[39–43]Our current study confirmed that that glycolysis is rapidly enhanced within 30 min after stimulation with TLR9 (CpG), which is the initial stimulus for the general activation of human naïve B cells (figure 2D–F, online supplemental figure S2D-F). TLR7 stimulation (Lox), which plays a critical role in SLE, was found to activate B cells (online supplemental figure S3), but did not enhance glycolysis. These findings suggest that different forms of TLR stimulation have distinct effects on B cells. Our study also confirmed that only glycolysis is enhanced in the early stage of human naïve B cell activation, whereas oxidative phosphorylation remains unaffected. CpG stimulation increased expression of the genes for glycolytic enzymes HK2, GAPDH and LDH in human naive B cells, without changes in the expression of PDH and PDK genes in the influx pathway to the TCA cycle, indicating that glycolytic metabolism does not depend on mitochondrial respiration. The current study is the first to provide a detailed evaluation of glycolytic enzyme gene expression in human naive B cells. TLR9 stimulation is involved in disease progression of SLE through the myeloid differentiation factor (MyD) 88 (MyD88), which occurs in the downstream metabolic and immune transcription pathways.[44] Furthermore, glycolysis in B cells was enhanced through MyD88.[45] Therefore, these previous studies support the current study, which showed that TLR9 stimulation enhanced glycolysis and aberrant B cell differentiation. B cells cause mitochondrial proliferation by enhancing glycolysis and increasing energy production by oxidative

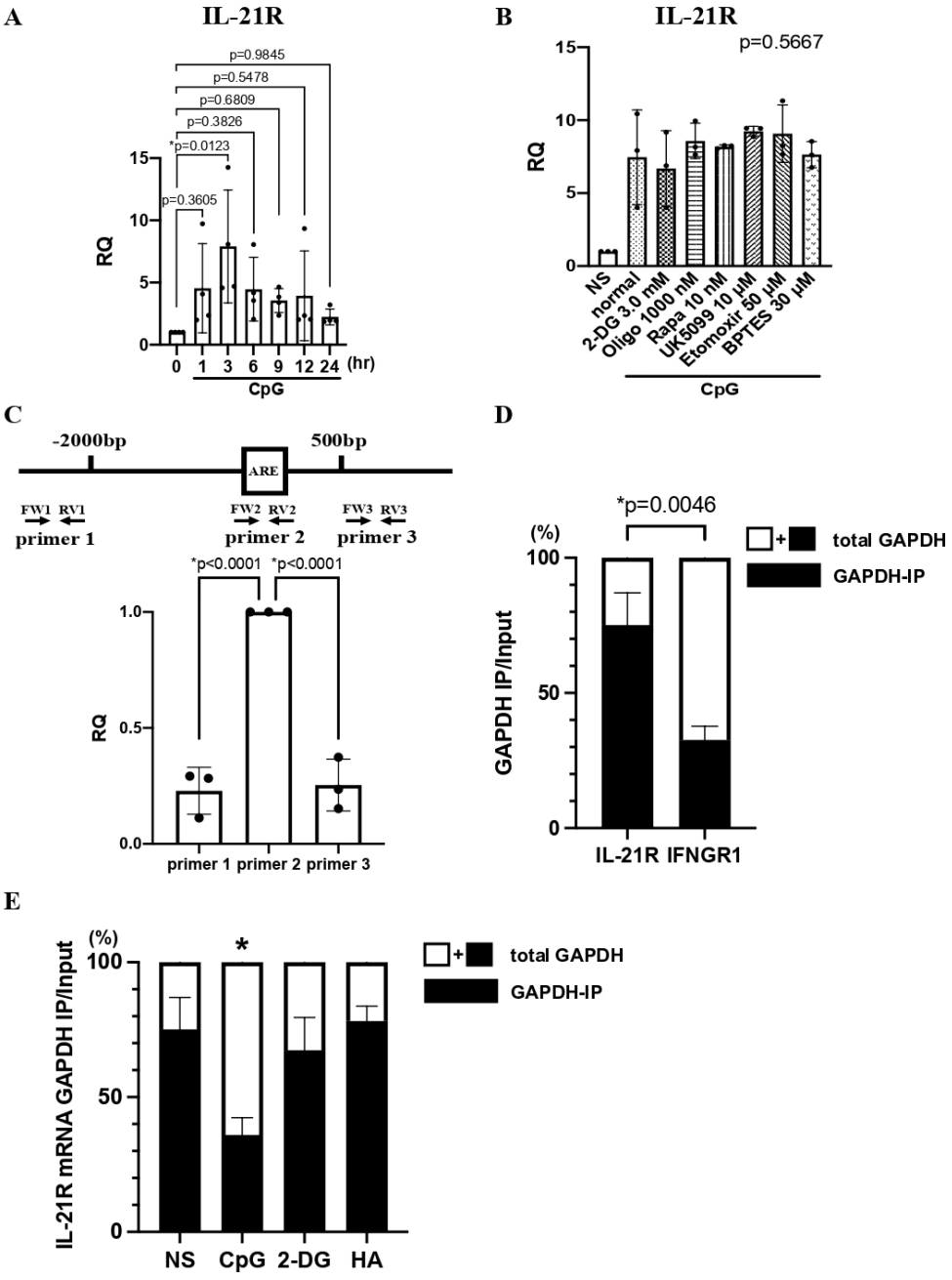

Figure 5 GAPDH binding to IL-21R mRNA inhibits protein translation IL-21R gene expression in CD19$^+$CD27$^-$ B cells (naïve B cells) and was measured by RIP-PCR using the anti-GAPDH antibody. (A) IL-21R gene expression in CD19$^+$CD27$^-$ B cells (naïve B cells) was measured using RT-PCR at 1, 3, 6, 9, 12 and 24 hours after CpG stimulation (n=4). (B) After pretreatment with each metabolic inhibitor for 30 min followed by culture with CpG stimulation for 3 hours, IL-21R gene expression in CD19$^+$CD27$^-$ B cells (naïve B cells) was measured by RT-PCR (n=3). (C) Comparison of the gene expression levels determined by RIP-PCR using primers 1 to 3 for IL-21R mRNA (n=3). (D) Comparison of the gene expression of IL-21R mRNA and IFNGR1 mRNA determined by RIP-PCR (n=3). Black bars (GAPDH-IP) show percentages of IL-21R and IFNGR1 mRNA captured by anti-GAPDH antibody during RNA-IP, relative to total RNA as determined from input (black bar+white bar). (E) Comparison of IL-21R mRNA gene expression determined using RIP-PCR among the conditions of no stimulation, CpG stimulation and CpG stimulation after pretreatment with 2-DG and HA (n=3). Black bars (GAPDH-IP) show the percentage of IL-21R mRNA captured by anti-GAPDH antibody during RNA-IP, relative to total RNA as determined from input (black bar+white bar). (A) The bars indicate the mean±SD from four independent experiments using CD19$^+$CD27$^-$ B cells (naïve B cells) from healthy controls. (B)–(E) The bars indicate the mean±SD from three independent experiments using CD19$^+$CD27$^-$ B cells (naïve B cells) from healthy controls. *P* values were determined using the Dunnett test (**A,** E), analysis of variance test (B), or Student's t-test (**C,** D). *p<0.05. NS, not stimulated; BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulphide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HA, heptelidic acid; IFNGR1, interferon γ receptor 1; IL-21R, interleukin 21 receptor; mRNA, messenger RNA; RIP, RNA immunoprecipitation; IP, immunoprecipitation, RQ, relative quantification; RTPCR, real-time PCR.

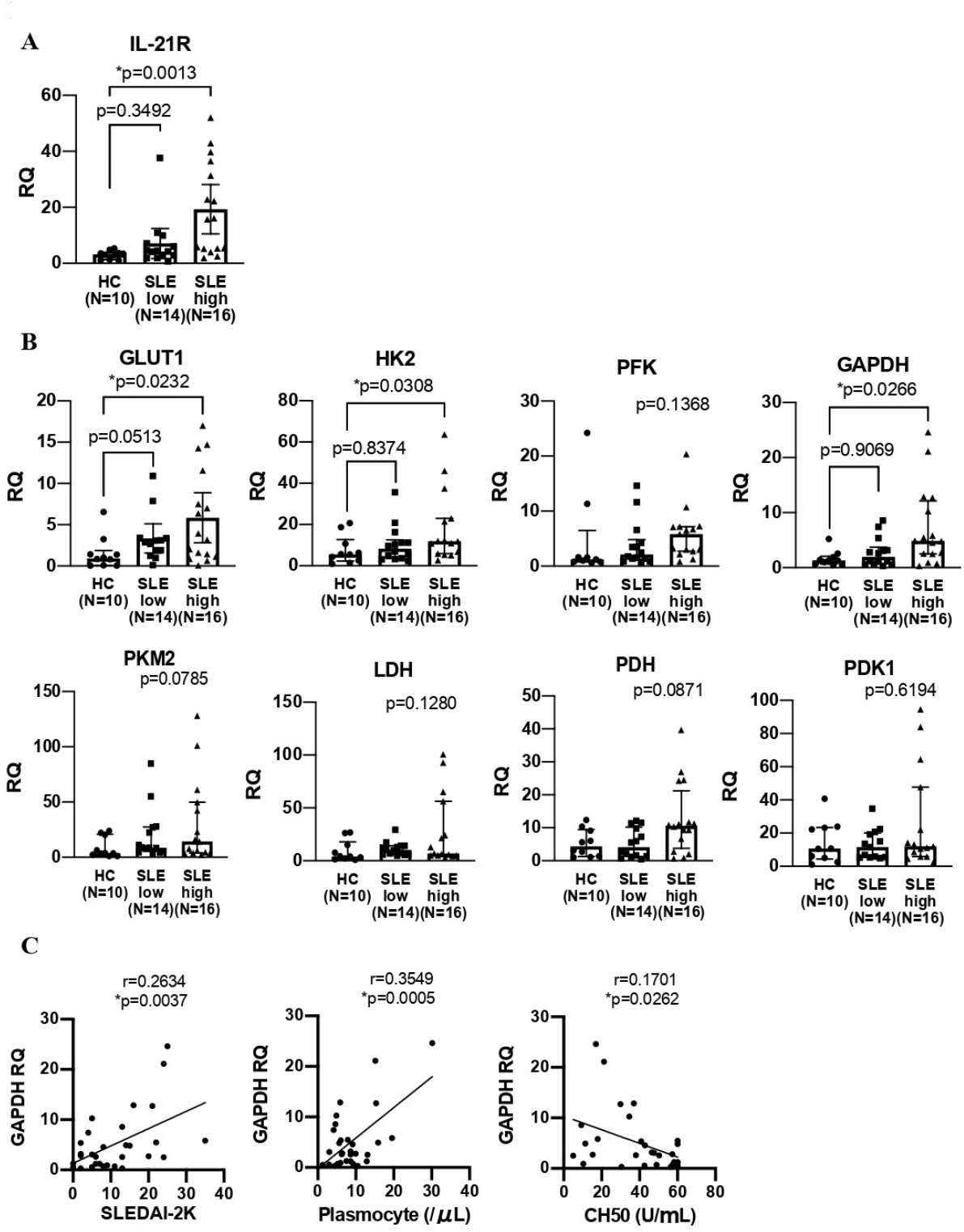

Figure 6 GAPDH levels increase in SLE patients with high disease activity and correlate with disease activity $CD19^{+}CD27^{-}$ B cells and were isolated from HCs and SLE patients. Metabolic function was evaluated with a flux analyser. The gene expression was evaluated using the RT-PCR. (A) Comparison of the gene expression level of IL-21R among HCs and SLE patients with low and high disease activity. (B) Comparison of the gene expression levels of GLUT1, HK2, PFK, GAPDH, PKM2, LDH, PDH and PDK1 in $CD19^{+}CD27^{-}$ B cells among HCs and SLE patients with low and high disease activity. (C) Correlation between GAPDH gene expression in $CD19^{+}CD27^{-}$ B cells and the clinical features of SLE patients. (A), (B) The bars indicate the mean±SD from healthy controls and SLE patients. $^{*}p<0.05$. HC, healthy control; CH50, 50% haemolytic complement activity; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT1, glucose transporter 1; HK2, hexokinase 2; IL-21R, interleukin 21 receptor; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PDK1, phosphoinositide-dependent kinase 1; PFK, phosphofructokinase; PKM2, pyruvate kinase M2; RQ, relative quantification; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000.

phosphorylation, eventually producing a substantial amount of energy needed to produce copious amounts of autoantibodies.[25 26] Early enhancement of glycolysis is a crucial precursor of B cell proliferation, differentiation and antibody production.

GAPDH performs other functions besides its primary role of a glycolytic enzyme. It is part of the group of 'moonlighting proteins', which are proteins with secondary functions.[28–31] It has been reported that the release of binding between GAPDH and mRNA increases protein expression involved in the mechanisms of IFN-γ production in $CD4^+$ T cells and TNF-α production in monocytes.[28 31] The current study demonstrated that the dissociation of the binding between GAPDH and IL-21R mRNA is involved in the regulation of IL-21R expression (figure 5E). In contrast, IFNGR1 mRNA had a low binding affinity for GAPDH, and protein expression was not inhibited by glycolytic inhibitors (figure 5D, online supplemental figure S4). Therefore, our findings suggested that, when glycolysis is enhanced, GAPDH bound to IL-21R mRNA in the nucleus is dissociated from IL-21R mRNA and migrates to the cytoplasm to function as a glycolytic enzyme, thereby eliminating inhibition of protein translation and enhancing protein expression. In fact, the expression level of GAPDH was increased in $CD19^+CD27^-$ naïve B cells of patients with SLE and was correlated with the levels of IL-21R, the plasmocyte count and the SLEDAI-2K. These results could also be attributed to the increase in cytoplasmic GAPDH levels and the subsequent enhancement of glycolysis. The abovementioned findings suggested that activated B cells not only produce energy necessary for proliferation and differentiation by rapidly enhancing glycolysis but may also enhance the expression of IL-21R, which is involved in B cell differentiation, and aberrant B cell differentiation.

The current study had some limitations. First, there is a limit to the number of cells isolated from the peripheral blood of patients with SLE. It would have been preferable to evaluate the metabolic functions and gene expression of each B cell subset individually, but due to limitations with respect to cell numbers, this was not possible. Consequently, we were unable to perform detailed analyses of the mechanisms in B cells derived from SLE patients, especially immunoprecipitation and other experiments. Determining whether the increase in GAPDH gene expression in naïve B cells from patients with SLE occurred in the cytoplasm or nucleus was not possible. Advances in techniques that will permit analysis of even a small number of cells in the future are anticipated. Second, many patients analysed in the current study had already received therapeutic interventions with glucocorticoids and immunosuppressive agents. In the future, studies enrolling patients with newly developed and treatment-naïve SLE are needed. Third, we were unable to absolutely conclude whether inhibition of GAPDH in B cells directly leads to suppression of SLE pathogenesis based on the results of the current study, which was mainly conducted in vitro with patient samples. In vivo studies using mice are needed to validate this finding in the future. Fourth, we did not acquire direct evidence that GAPDH inhibits the translation of IL-21 mRNA into protein. Ideally, experiments with GAPDH knockout cells would have been conducted, but due to limited cell numbers, this was not feasible.

The current study revealed the intracellular metabolic mechanism in the early stage of B cell activation, which triggers B cell activation and disease progression in patients with SLE. Enhanced glycolysis may be a new marker for the pathological condition in exacerbating SLE.

Author affiliations
[1]The First Department of Internal Medicine, University of Occupational and Environmental Health Japan, Kitakyushu, Japan
[2]Department of Rheumatology and Clinical Immunology, Wakayama Medical University, Wakayama, Japan
[3]Department of Molecular Targeted Therapies, University of Occupational and Environmental Health Japan, Kitakyushu, Japan
[4]Department of Clinical Nursing, University of Occupational and Environmental Health Japan, Kitakyushu, Japan

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ORCID iDs
Masanobu Ueno http://orcid.org/0009-0003-0212-172X
Shigeru Iwata http://orcid.org/0000-0002-2076-1603
Koshiro Sonomoto http://orcid.org/0000-0002-4003-8842
Satoshi Kubo http://orcid.org/0000-0001-9693-9263
Yusuke Miyazaki http://orcid.org/0000-0001-5692-3881
Ryuichiro Kanda http://orcid.org/0000-0001-8247-6595
Yoshiya Tanaka http://orcid.org/0000-0002-0807-7139

REFERENCES

1 Tanaka Y, Kubo S, Iwata S, *et al*. B cell phenotypes, signaling and their roles in secretion of antibodies in systemic lupus erythematosus. *Clin Immunol* 2018;186:21–5.
2 Tanaka Y. Systemic lupus erythematosus. *Best Pract Res Clin Rheumatol* 2022;36:101814.
3 Fanouriakis A, Tziolos N, Bertsias G, *et al*. Update on the diagnosis and management of systemic lupus erythematosus. *Ann Rheum Dis* 2021;80:14–25.
4 Parodis I, Long X, Karlsson MCI, *et al*. B Cell Tolerance and Targeted Therapies in SLE. *J Clin Med* 2023;12:6268.
5 Yuan Y, Zhao L, Ye Z, *et al*. Association of toll-like receptor 9 expression with prognosis of systemic lupus erythematosus. *Exp Ther Med* 2019;17:3247–54.
6 Rao H, Zeng Q, Liang Y, *et al*. Correlation between TLR9 Expression and Cytokine Secretion in the Clinical Diagnosis of Systemic Lupus Erythematosus. *Mediators Inflamm* 2015;2015:710720.
7 Elloumi N, Fakhfakh R, Abida O, *et al*. Relevant genetic polymorphisms and kidney expression of Toll-like receptor (TLR)-5 and TLR-9 in lupus nephritis. *Clin Exp Immunol* 2017;190:328–39.
8 Iwata S, Yamaoka K, Niiro H, *et al*. Amplification of Toll-like receptor-mediated signaling through spleen tyrosine kinase in human B-cell activation. *J Allergy Clin Immunol* 2012;129:1594–601.
9 Kitanaga Y, Yamajuku D, Kubo S, *et al*. Discovery of a novel Igβ and FcγRIIB cross-linking antibody, ASP2713, and its potential application in the treatment of systemic lupus erythematosus. *Int Immunopharmacol* 2021;101:108343.
10 Yoshikawa M, Nakayamada S, Kubo S, *et al*. Type I and II interferons commit to abnormal expression of chemokine receptor on B cells in patients with systemic lupus erythematosus. *Clin Immunol* 2019;200:1–9.
11 Kubo S, Nakayamada S, Yoshikawa M, *et al*. Peripheral Immunophenotyping Identifies Three Subgroups Based on T Cell Heterogeneity in Lupus Patients. *Arthritis Rheumatol* 2017;69:2029–37.
12 Morel L. Immunometabolism in systemic lupus erythematosus. *Nat Rev Rheumatol* 2017;13:280–90.
13 Yin Y, Choi S-C, Xu Z, *et al*. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* 2015;7:274ra18.
14 Yin Y, Choi S-C, Xu Z, *et al*. Glucose Oxidation Is Critical for CD4+ T Cell Activation in a Mouse Model of Systemic Lupus Erythematosus. *J Immunol* 2016;196:80–90.
15 Gubser PM, Bantug GR, Razik L, *et al*. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* 2013;14:1064–72.
16 Angiari S, Runtsch MC, Sutton CE, *et al*. Pharmacological Activation of Pyruvate Kinase M2 Inhibits $CD4^+$ T Cell Pathogenicity and Suppresses Autoimmunity. *Cell Metab* 2020;31:391–405.
17 Murray PJ, Rathmell J, Pearce E. SnapShot: Immunometabolism. *Cell Metab* 2015;22:190.
18 Wu C, Fu Q, Guo Q, *et al*. Lupus-associated atypical memory B cells are mTORC1-hyperactivated and functionally dysregulated. *Ann Rheum Dis* 2019;78:1090–100.
19 Raza IGA, Clarke AJ. B Cell Metabolism and Autophagy in Autoimmunity. *Front Immunol* 2021;12:681105.
20 Sharabi A, Tsokos GC. T cell metabolism: new insights in systemic lupus erythematosus pathogenesis and therapy. *Nat Rev Rheumatol* 2020;16:100–12.
21 Perl A. Review: Metabolic Control of Immune System Activation in Rheumatic Diseases. *Arthritis Rheumatol* 2017;69:2259–70.
22 Robinson GA, Wilkinson MGL, Wincup C. The Role of Immunometabolism in the Pathogenesis of Systemic Lupus Erythematosus. *Front Immunol* 2021;12:806560.
23 Takeshima Y, Iwasaki Y, Nakano M, *et al*. Immune cell multiomics analysis reveals contribution of oxidative phosphorylation to B-cell functions and organ damage of lupus. *Ann Rheum Dis* 2022;81:845–53.
24 Iwata S, Hajime Sumikawa M, Tanaka Y. B cell activation via immunometabolism in systemic lupus erythematosus. *Front Immunol* 2023;14:1155421.
25 Sumikawa MH, Iwata S, Zhang M, *et al*. An enhanced mitochondrial function through glutamine metabolism in plasmablast differentiation in systemic lupus erythematosus. *Rheumatology (Oxford)* 2022;61:3049–59.
26 Zhang M, Iwata S, Hajime M, *et al*. Methionine Commits Cells to Differentiate Into Plasmablasts Through Epigenetic Regulation of BTB and CNC Homolog 2 by the Methyltransferase EZH2. *Arthritis Rheumatol* 2020;72:1143–53.
27 Soliman GA, Abzalimov RR, He Y. mTORC1 and mTORC2 Complexes Regulate the Untargeted Metabolomics and Amino Acid Metabolites Profile through Mitochondrial Bioenergetic Functions in Pancreatic Beta Cells. *Nutrients* 2022;14:3022.
28 Chang CH, Curtis JD, Maggi LB Jr, *et al*. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 2013;153:1239–51.
29 Nicholls C, Li H, Liu JP. GAPDH: a common enzyme with uncommon functions. *Clin Exp Pharmacol Physiol* 2012;39:674–9.
30 Harada N, Yasunaga R, Higashimura Y, *et al*. Glyceraldehyde-3-phosphate dehydrogenase enhances transcriptional activity of androgen receptor in prostate cancer cells. *J Biol Chem* 2007;282:22651–61.
31 Millet P, Vachharajani V, McPhail L, *et al*. GAPDH Binding to TNF-α mRNA Contributes to Posttranscriptional Repression in Monocytes: A Novel Mechanism of Communication between Inflammation and Metabolism. *J Immunol* 2016;196:2541–51.
32 Caro-Maldonado A, Wang R, Nichols AG, *et al*. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J Immunol* 2014;192:3626–36.
33 Jellusova J, Cato MH, Apgar JR, *et al*. Gsk3 is a metabolic checkpoint regulator in B cells. *Nat Immunol* 2017;18:303–12.
34 Zhang X, Yang Y, Jing L, *et al*. Pyruvate Kinase M2 Contributes to TLR-Mediated Inflammation and Autoimmunity by Promoting Pyk2 Activation. *Front Immunol* 2021;12:680068.
35 Masoumi M, Alesaeidi S, Khorramdelazad H, *et al*. Role of T Cells in the Pathogenesis of Rheumatoid Arthritis: Focus on Immunometabolism Dysfunctions. *Inflammation* 2023;46:88–102.
36 Ge T, Yang J, Zhou S, *et al*. The Role of the Pentose Phosphate Pathway in Diabetes and Cancer. *Front Endocrinol (Lausanne)* 2020;11:365.
37 Jung J, Zeng H, Horng T. Metabolism as a guiding force for immunity. *Nat Cell Biol* 2019;21:85–93.
38 Maciolek JA, Pasternak JA, Wilson HL. Metabolism of activated T lymphocytes. *Curr Opin Immunol* 2014;27:60–74.
39 Boothby M, Rickert RC. Metabolic Regulation of the Immune Humoral Response. *Immunity* 2017;46:743–55.
40 Akkaya M, Traba J, Roesler AS, *et al*. Second signals rescue B cells from activation-induced mitochondrial dysfunction and death. *Nat Immunol* 2018;19:871–84.
41 Torigoe M, Iwata S, Nakayamada S, *et al*. Metabolic Reprogramming Commits Differentiation of Human CD27+IgD+ B Cells to Plasmablasts or CD27−IgD− Cells. *J Immunol* 2017;199:425–34.
42 Elkon KB, Briggs TA. The (Orf)ull truth about IRF5 and type I interferons in SLE. *Nat Rev Rheumatol* 2020;16:543–4.
43 Heinz LX, Lee J, Kapoor U, *et al*. TASL is the SLC15A4-associated adaptor for IRF5 activation by TLR7-9. *Nature New Biol* 2020;581:316–22.
44 Leibler C, John S, Elsner RA, *et al*. Genetic dissection of TLR9 reveals complex regulatory and cryptic proinflammatory roles in mouse lupus. *Nat Immunol* 2022;23:1457–69.
45 Sintes J, Gentile M, Zhang S, *et al*. mTOR intersects antibody-inducing signals from TACI in marginal zone B cells. *Nat Commun* 2017;8:1462.