Overexpression of FcγRIIB regulates downstream protein phosphorylation and suppresses B cell activation to ameliorate systemic lupus erythematosus

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Abstract. The present study aimed to examine the effects of FcγRIIB on systemic lupus erythematosus (SLE) and to investigate the underlying mechanisms. For this purpose, lentiviral vector carrying the membrane-bound type FcγRIIB gene (mFcγRIIB lentivirus) and soluble FcγRIIB (sFcγRIIB) protein were used to treat B cells from patients with SLE. The B cells were treated with calf thymus DNA (ctDNA) and anti-calf thymus DNA-immune complexes (anti-ctDNA-IC). mFcγRIIB lentivirus and sFcγRIIB protein were also injected into MRL/lpr SLE mice. The results revealed that anti-ctDNA-IC treatment significantly downregulated the IgG antibody secretion of B cells treated with mFcγRIIB lentivirus. mFcγRIIB and sFcγRIIB decreased the phosphorylation level of Bruton's tyrosine kinase (BTK) in B cells, and increased the phosphorylation level of Lyn proto-oncogene (Lyn), docking protein 1 (DOK1) and inositol polyphosphate-5-phosphatase D (SHIP). mFcγRIIB promoted the apoptosis of B cells. Following the treatment of MRL/lpr SLE mice with mFcγRIIB lentivirus, the levels of urinary protein, serum anti-nuclear and anti-dsDNA antibodies were decreased, while the levels of mFcγRIIB in B cells were increased. mFcγRIIB ameliorated the pathologies of the kidneys, liver and lymph node tissues of the MRL/lpr SLE mice. Following treatment of the MRL/lpr SLE mice with sFcγRIIB, the levels of urinary protein, serum anti-dsDNA antibody and BTK and SHIP phosphorylation levels in B cells were decreased, while the serum sFcγRIIB and sFcγRIIB-IgG levels were increased. On the whole, the findings of the present study demonstrate that recombinant FcγRIIB inhibits the secretion of IgG antibody by B cells from patients with SLE, ameliorates the symptoms of SLE in mice, and alters the phosphorylation levels of downstream proteins of the FcγRIIB signaling pathway in B cells. These results suggest that FcγRIIB may play preventive and therapeutic roles in SLE by inhibiting B cell activation via the FcγRIIB signaling pathway, which provides a novel theory and strategy for the prevention and treatment of SLE.

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Abbreviations: SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; RNP, ribonucleoproteins; CICs, circulating immune complexes; ctDNA, calf thymus DNA; ICs, immune complexes; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB; BTK, Bruton's tyrosine kinase; Lyn, Lyn proto-oncogene, Src family tyrosine kinase; DOK1, docking protein 1; SHIP, inositol polyphosphate-5-phosphatase D; BCR, B cell receptor

Key words: FcγRIIB, B cells, systemic lupus erythematosus, IgG antibody, Bruton's tyrosine kinase, Lyn, docking protein 1, inositol polyphosphate-5-phosphatase D

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects several organs, including the skin, joints, central nervous system and kidneys. The occurrence of SLE is related to hormonal, environmental and genetic factors; however, the pathogenesis of SLE requires further study (1,2). High titers of autoantibodies against double-stranded DNA (dsDNA) and ribonucleoproteins (RNP) are often detectable in patients with SLE several years before clinical manifestations arise. These autoantibodies can bind to autoantigens and complement factors to form circulating immune complexes (CICs), which are deposited in target organs to induce inflammation and cause various diseases (3).

As the main force of the immune response, B cells play an important role in the humoral immune response. However, studies have demonstrated that B lymphocytes acting as effector cells can increase the occurrence and chronic persistence of SLE (4,5). Signal transduction abnormity, tolerance deficiency and immunoregulatory mechanism abnormity in B cells, induced by environmental and genetic factors, play important roles in the occurrence and development of SLE (6,7).

FcγRIIB, as an immunoregulatory receptor, is expressed mainly on the surface of B cells. It contains the extracellular region with the Ig-like domain, transmembrane region and intracellular region with the immunoreceptor tyrosine inhibitory motif (ITIM), which has an inhibitory function. The extracellular domain is a low affinity binding IgG-Fc region. Antigens and cytokines can stimulate the hydrolysis of the extracellular domain of membrane-bound type FcγRIIB (mFcγRIIB) or the selective splicing of the FcγRIIB gene to produce soluble FcγRIIB (sFcγRIIB) (8). FcγRIIB can regulate healthy B cells, induces immune tolerance by inhibiting B cell expression of antibodies, and is a key inhibitory receptor mediating B cells activation (9,10). The inhibitory effect of FcγRIIB depends on the ITIM that is phosphorylated upon FcγRIIB coaggregation with ITAM-bearing receptors and recruits SH2 domain-containing phosphatases (11). The decrease in FcγRIIB expression exerts adverse effects on the immune response, leading to the occurrence of autoimmune diseases. The level of FcγRIIB on the surfaces of immature B cells and memory B cells in patients with autoimmune diseases has been found to be lower than that in healthy subjects (12,13), causing B lymphocytes to produce a large number of antibodies against autoantigens in a hyperimmune response. However, it has been reported that increasing the expression of FcγRIIB in B lymphocytes reduces the production of antibodies and immune complexes in lupus mice, and improves the symptoms of lupus (14). In transgenic mice with FcγRIIB overexpression on the surface of B lymphocytes, the production of T cell-dependent antibody IgG has been shown to be significantly reduced, and the symptoms of SLE are decreased (15). Therefore, increasing the FcγRIIB levels on the surface of B cells may be a promising approach for treating autoimmune diseases. Recombinant human sFcγRIIB can be used as a potential target for the treatment of autoimmune diseases mediated by antibodies and immune complexes. However, to the best of our knowledge, there are no available reports to date on the negative regulation of antibody secretion in patients with SLE by recombinant human sFcγRIIB. More importantly, the mechanisms of FcγRIIB in SLE remain unclear. In the present study, the effects of mFcγRIIB and sFcγRIIB proteins on the IgG antibody secretion of B cells form patients with SLE, and the preventative and therapeutic effects of sFcγRIIB in mice with SLE were examined. In addition, the underlying mechanisms were investigated by measuring the phosphorylation levels of Bruton's tyrosine kinase (BTK), DOK1, docking protein 1 (DOK1), Lyn proto-oncogene (Lyn) and inositol polyphosphate-5-phosphatase D (SHIP) in the downstream signaling pathways of B cell receptor (BCR) and FcγRIIB in B cells.

Materials and methods

Construction of mFcγRIIB lentivirus. Total RNA from was isolated human B cells (isolated from patients with SLE as described below) using TRIzol RNA extraction reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, cDNA was synthesized using 1 μg of total RNA and the reverse transcription system [Tiangen Biochemical Technology (Beijing) Co., Ltd.]. The FcγRIIB gene was cloned by nest PCR using Taq PCR MasterMix [Tiangen Biochemical Technology (Beijing) Co., Ltd.] and outer primers (forward primer, 5'-ATCCGCCAAGCTTTGAGAGAAGGCTGTGAC T-3' and reverse primer, 5'-AGGGAGCTTCAGGACTCA GGTAGATGACT-3') at 58˚C annealing temperature for the first PCR and inter primers (forward primer, 5'-GCCCCC GGGACGCGTATGGGAATCCTGTCATTCTTACC-3' and reverse primer, 5'-CTACCCGGTAGAATTCCTAAATAC GGTTCTGGTCATCAG-3') at 56˚C annealing temperature for the second PCR. The FcγRIIB gene was inserted into the lentivirus-induced expression vector pLVX-TRE3G-ZsGreen1 (Takara Bio, Inc.) with green fluorescent protein (GFP) gene to construct the mFcγRIIB lentivirus.

Lentivirus packaging. The constructed human mFcγRIIB lentivirus (10^6 TU/ml), mouse mFcγRIIB lentivirus (10^6 TU/ml) from our laboratory, and virus tetracycline (Tet) (10^5 TU/ml) regulatory plasmid were transfected into lenti-X 293T cells (Takara Bio, Inc.) to complete lentivirus packaging using a Lenti-X Lentiviral packaging system (Takara Bio, Inc.), according to the manufacturer's instructions.

HT-1080 cell culture. HT-1080 cells were purchased from the Shanghai Institutes for Biological Sciences, and were cultured in Dulbecco's modified Eagle's medium (HyClone; GE Healthcare Life Sciences) supplemented with 10% (v/v) fetal bovine serum (HyClone; GE Healthcare Life Sciences) and penicillin-streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in a 95% O_2 and 5% CO_2 incubator at 37˚C.

Establishment of HT-1080 cells stably expressing mFcγRIIB. A total of $2x10^5$ HT-1080 cells were infected with lentivirus regulatory plasmid, followed by the addition of 1 ml of polyethylene (10 mg/ml) virus diluent (diluted 6 times) to HT-1080 cells for infection, incubate at 37˚C, 5% CO_2 for 12 h, and then add G418 (100 μg/μl) or Puro (0.3 μg/μl) working solution, G418 (200 μg/ml) was used to screen single G418-resistant HT-1080 cells. Subsequently, scale-up cultured G418-resistant HT-1080 cells were infected with mFcγRIIB lentivirus, and single G418- and Puro-resistant HT-1080 cells were then screened using Puro (0.3 μg/ml, Takara Bio, Inc.), cultured at 37˚C, 5% CO_2 for 7-14 days; the viral titer was $4x10^7$ TU/ml.

Immunofluorescence assay. G418- and Puro-resistant HT-1080 cells were inoculated into 6-well plates, and inducer doxycycline (Dox) (Takara Bio, Inc.) was added to induce the cells at a final concentration of 1,000 ng/ml. The cells were then incubated for 48 h at 37˚C with 5%CO_2. After washing with PBS, PE-labeled anti-FcγRIIB antibody (cat. no. 563019; 5 μl; BD Biosciences) was incubated with the cells for 20 min on ice and in the dark. After washing with PBS, cells were observed under transmission light, green fluorescence and red fluorescence microscopes (Olympus Corporation).

Western blot analysis. Double-resistant HT-1080 cells were induced by Dox inducer at concentrations of 100, 500, 1,000 ng/ml and incubated for 48 h at 37˚C with 5% CO_2. Total protein was extracted from the cells using lysis buffer (KeyGEN BioTECH). After determining the protein concentration using the BCA analysis kit (KeyGEN BioTECH), an equal amount of proteins (100 ng) were separated by 10%

SDS-PAGE, and then transferred to polyvinylidene difluoride membranes, which were blocked with 5% skim milk in TBS with 1% Tween-20 for 90 min at 25˚C. Subsequently, the membranes were probed with primary antibodies at 4˚C overnight, followed by incubation with secondary antibodies for a further 2 h at room temperature, each sample of total protein was assayed by western blot analysis using mouse monoclonal anti-human FcγRIIB antibody (cat. no. sc-166711; 1:2,000; Santa Cruz Biotechnology, Inc.) and peroxidase-conjugated rabbit anti-mouse IgG antibody (cat. no. sc-2357; 1:2,000; Santa Cruz Biotechnology, Inc.) (16). The immunoreactive bands were visualized using an enhanced chemiluminescent (ECL) kit (KeyGEN BioTECH). The blots were analyzed using ImageJ 1.48u software (National Institutes of Health). β-actin (cat. no. sc-81178; 1:500; Santa Cruz Biotechnology, Inc.) was used as the loading control.

Preparation of recombinant sFcγRIIB. In order to prepare human recombinant sFcγRIIB, PET-sFcγRIIB plasmid from our laboratory was transformed into competent *Escherichia coli* BL21 cells (Takara Bio, Inc.). To prepare mouse recombinant sFcγRIIB, mouse sFcγRIIB gene was cloned using TRE-sFcγRIIB plasmid as a template and sFcγRIIB primers (forward primer, 5'-GGAATTCATGGGAATCCTGCCGTT CCTACTGA-3' and reverse primer, 5'-CCCAAGCTTTGT CAATACTGGTAAAGACCTGCTG-3'), and was inserted into pET-32a (+) (Beijing Solarbio Science & Technology Co., Ltd.) vector to construct the PET-sFcγRIIB plasmid. The PET-sFcγRIIB plasmid (2 μl) was subsequently was transformed into competent *Escherichia coli* BL21 cells. Both sets of transformed BL21 cells were induced by IPTG to produce sFcγRIIB proteins, which were purified using a His Bind® Purification kit (Merck & Co., Inc.) according to the manufacturer's instructions. Purified sFcγRIIB proteins were validated by western blot analysis.

ELISA for sFcγRIIB and IgG. A total of 30 female patients with SLE (35±10 years old) from the Department of Rheumatism, General Hospital of Ningxia Medical University were recruited into the present study based on the American College of Rheumatology (ACR) criteria (17), and 30 healthy female subjects (37±8 years old) from the Cardiovascular Hospital of Ningxia Medical University were also recruited (October, 2018 to March, 2019). All subjects signed informed consent froms prior to the start of the study. The present study was approved by the Ethics Committee of Ningxia Medical University. Serum was collected from all subjects. For serum sFcγRIIB detection, mouse anti-human sFcγRIIB monoclonal antibody (cat. no. sc-166578; 1:2,000; Santa Cruz Biotechnology, Inc.), serum (1:100; Biological Industries), rabbit anti-human sFcγRIIB polyclonal antibody (cat. no. bs-4991R;1:2,000; Beijing Bioss Biotechnology Co., Ltd.) and HRP-labeled anti-IgG (cat. no. bs-0297G-HRP;1:8,000; Beijing Bioss Biotechnology Co., Ltd.) were successively added into the plates for incubation (37˚C, 30 min). For serum sFcγRIIB-IgG determination, mouse anti-human sFcγRIIB monoclonal antibody (cat. no. bs-4991R; 1:2,000; Beijing Bioss Biotechnology Co., Ltd.), serum (1:100; Biological Industries), and HRP-labeled anti-IgG (cat. no. bs-0297G-HRP;1:8,000; Beijing Bioss Biotechnology Co., Ltd.) were successively incubated in plates (37˚C, 30 min). For the binding strength detection of recombinant human sFcγRIIB to IC in serum, serum treated with 5 μg/ml calf thymus DNA (ctDNA; Sigma-Aldrich; Merck KGaA) and HRP-labeled anti-IgG (cat. no. bs-40295G-HRP; 1:8,000; Beijing Bioss Biotechnology Co., Ltd.) were successively incubated (37˚C, 30 min) in plates previously coated with 1.25 μg/ml recombinant sFcγRIIB. Following these incubations, TMB reagent was added to all plates for chromogenic reaction. The optical density was determined using a microplate reader (ELx800NB, BioTek Instruments, Inc.).

B cell preparation. Peripheral blood samples were collected from all study participants and the lymphocytes were isolated with lymphocyte separation liquid. A magnetic bead separation system (Miltenyi Biotec GmbH) was used to sort $CD19^+$ B cells with anti-$CD19^+$ antibody (cat. no. 130-113-733; 1:50; Miltenyi Biotec GmbH). Sorted $CD19^+$ B cells were cultured for scale-up.

B cell treatment. For mFcγRIIB treatment, mFcγRIIB lentivirus, lentivirus regulatory plasmid and Dox were added to the $CD19^+$ B cells. The expression of mFcγRIIB in the treated B cells was then detected by immunofluorescence assay and western blot analysis according to the procedures described above. Subsequently, ctDNA and anti-ctDNA-IC (the mixture of SLE patient serum and ctDNA at a final concentration of 5 μg/ml, incubated at 37˚C for 1 h) were incubated with treated $CD19^+$ B cells. After 24 h, the B cells were collected for flow cytometry. Briefly, the collected cells were washed twice with ice-cold PBS at pH 7.4, and were then resuspended in buffer containing Annexin V (Elabscience); at a concentration of $1x10^6$/ml cells/ml. Subsequently, 5 μl Alexa Fluor 555 (Thermo Fisher Scientific, Inc.) and 1 μl 100 μg/ml PI (Thermo Fisher Scientific, Inc.) were added to 100 μl cell suspension. After gentle vortexing, the cells were incubated at 4˚C in the dark for 15 min and analyzed by flow cytometry (Merck KGaA). The percentages of positively stained cells were determined. For sFcγRIIB treatment, recombinant sFcγRIIB and ctDNA were used to treat $CD19^+$ B cells for 72 h. Cell medium from both virus and protein treatments was collected for IgG detection using an ELISA kit (eBioscience), according to the manufacturer's instructions. B cells were collected for the determination of the BTK, Lyn, DOK1 and SHIP protein and phosphorylation levels by western blot analysis using antibodies against BTK (cat. no. ab208937; 1:500; Abcam), Lyn (cat. no. ab1890; 1:1,000; Abcam), DOK1 (cat. no. ab8112; 1:1,000; Abcam), SHIP (cat. no. ab45142; 1:1,000; Abcam), phosphorylated BTK (cat. no. ab68217; 1:1,000; Abcam), phosphorylated DOK1 (cat. no. ab75742; 1:1,000; Abcam), phosphorylated Lyn (cat. no. ab33914; 1:1,000; Abcam) and phosphorylated SHIP (cat. no. ab96402; 1:1,000; Abcam), the membranes were probed with primary antibodies at 4˚C overnight, followed by incubation with secondary antibodies for a further 2 h at room temperature.

Animal experiments. A total of 160 MRL/lpr SLE model mice (all female mice) with a body weight of 16-20 g were purchased from the Experimental Animal Center of Nanjing Military Region (no. 0032260). All animals were kept in cages with a light/dark cycle of 12 h at 25±1˚C, with free access

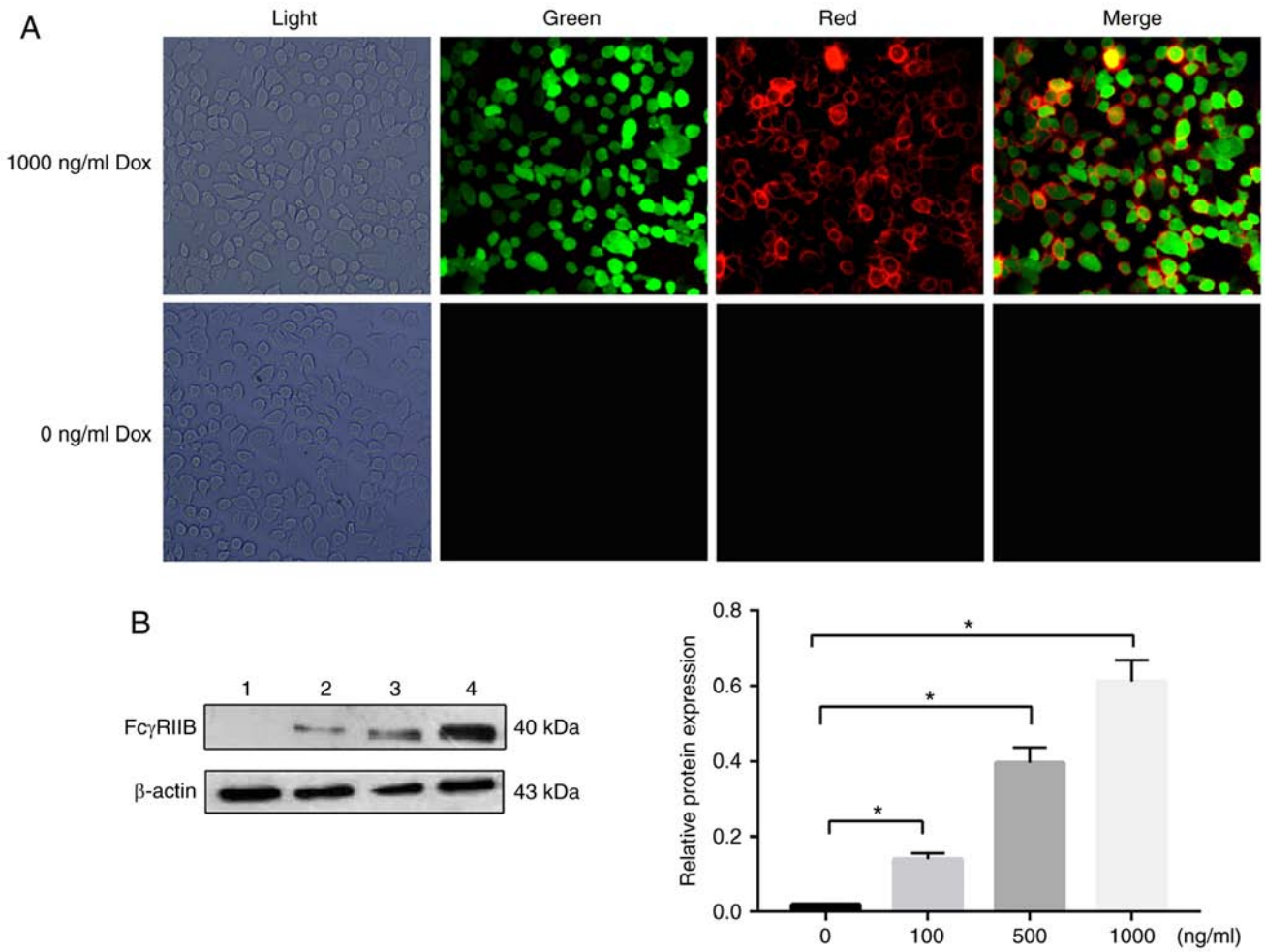

Figure 1. (A) Observation of double-resistant HT-1080 cells by fluorescence microscopy (magnification x400). The expression of human mFcγRIIB in HT-1080 cells was detected by immunofluorescence staining. Light, light field; green, green fluorescent protein (GFP) expression in cells; red, mFcγRIIB expression in cells; merge, GFP and mFcγRIIB expression in cells. The experiments were repeated 3 times. (B) The protein expression levels of sFcγRIIB in human were examined by western blot analysis, with β-actin as a loading control. Lane 1, 0 ng/ml Dox inducer; lane 2, 100 ng/ml Dox inducer; lane 3, 500 ng/ml Dox inducer; lane 4, 1,000 ng/ml Dox inducer. *P<0.05. Data are presented as the means ± SD of 3 independent experiments. Dox, doxycycline; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB.

to food and water; the health and behavior of the mice were monitored every 5 days. Among the 160 mice, mice (8 weeks old) that had no lupus symptoms were designated as the prevention group (n=80/group) and 16-week-old mice that had obvious lupus symptoms were designated as the treatment group (n=80/group). The animal experiments were approved by the Animal Ethics Committee of Ningxia Medical University and followed the Guidelines for the Management and Use of Laboratory Animals (National Academies Press). For mFcγRIIB treatment, the prevention (pre) and treatment (tre) groups were treated with 100 μl or 200 μl suspensions of mFcγRIIB lentivirus, lentivirus regulatory plasmid and Dox, 100 μl suspensions of lentivirus empty vector, and healthy saline via the tail vein (n=10/group). For sFcγRIIB treatment, the prevention group and treatment groups were treated with intravenous 4.8 μg (60 μl), 9.6 μg (120 μl), and 14.4 μg (180 μl) recombinant mouse FcγRIIB and normal saline, once per week for 4 consecutive weeks (n=10/group). Following observation for a week, serum and urine were collected, and the mice were then anesthetized by an intraperitoneal injection of chloral hydrate (4%, 400 mg/kg) and were sacrificed by cervical dislocation. Mouse death was confirmed by the inexistence of breath, heartbeat and corneal reflex. No accidental deaths occurred during the experiment.

Kidney, liver and lymph tissues were obtained for H&E staining. The mice were sacrificed and the kidney, liver and lymph tissues of the mice were obtained and fixed in 4% (v/v) paraformaldehyde, embedded in paraffin and sectioned at a 5-8 μm thickness. The fixed kidney, liver and lymph tissues were stained with hematoxylin and eosin (H&E) for the evaluation of the severity of kidney, liver and lymph injury. The samples were dewaxed with xylene (cat. no. 10023428; Sinopharm Chemical Reagent Co., Ltd.) and then dephenylated using a graded ethanol series (100, 95, 80 and 70%) for 2 min. The tissues were then rehydrated by rinsed in distilled water twice to, stained using 0.5% hematoxylin (cat. no. G1120; Solarbio Life Science Co., Ltd.) for 20 min at room temperature and then washed under running water. The slices were then rinsed in acidification solution comprised of hydrochloric acid (cat. no. 10011018; Sinopharm Chemical Reagent Co., Ltd.) and 75% ethanol for 30 sec and then washed with tap water for 15 min. The tissues were then stained using

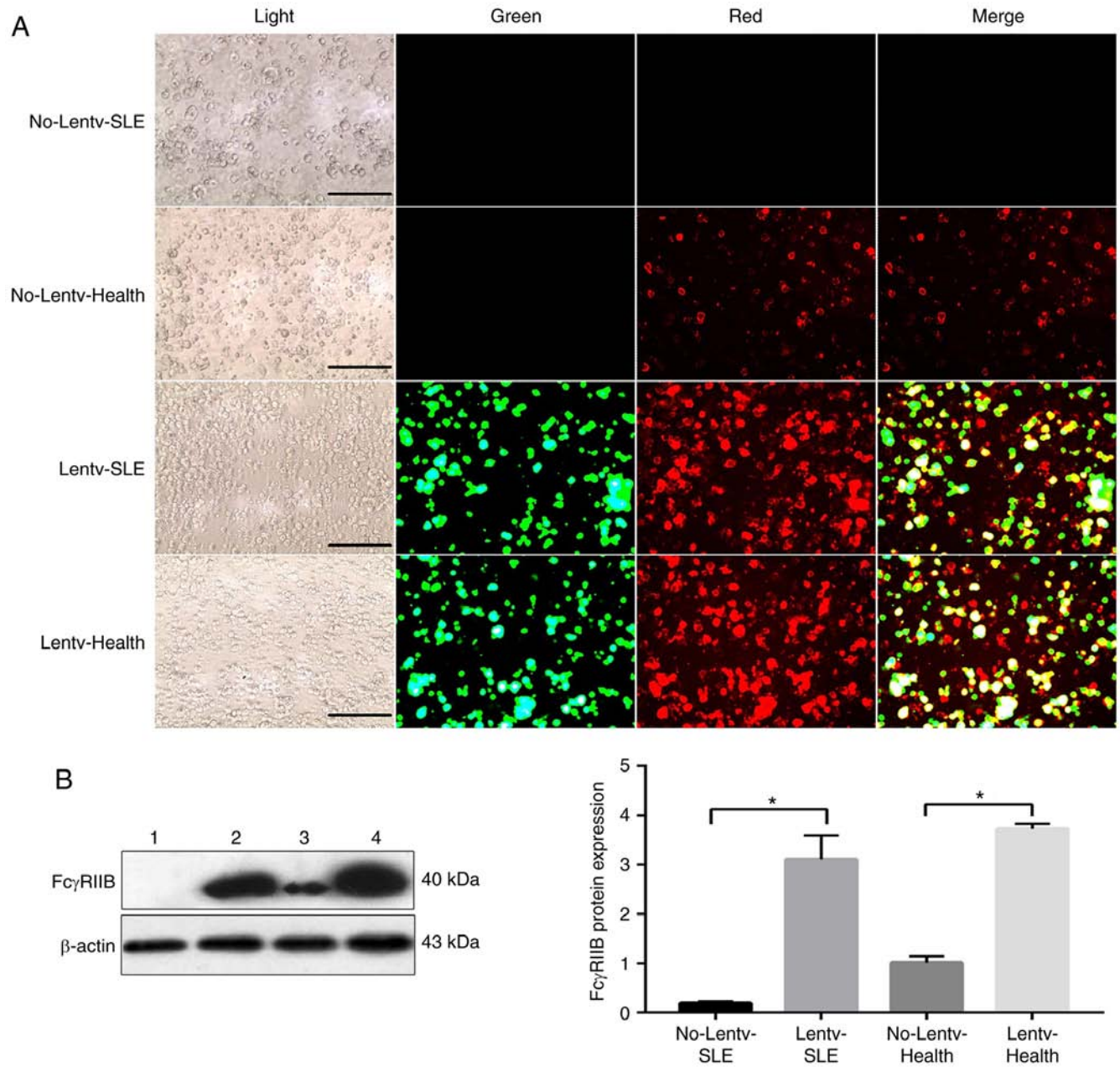

Figure 2. (A) Fluorescence microscopy to observe the expression of mFcγRIIB in B lymphocytes (magnification 400x). B cells were transfected using mFcγRIIB-lentivirus for 72 h. The infection efficiency of mFcγRIIB-lentivirus was observed by fluorescence microscopy. No-Lentv-SLE, no transfection in the SLE patients; Lentv-SLE, mFcγRIIB-lentivirus transfection in the SLE patients; No-Lentv-health, no transfection in the healthy controls; Lentiv-health, mFcγRIIB-lentivirus transfection in the healthy controls. Light, light field; green, green fluorescent protein (GFP) expression in cells; red, mFcγRIIB expression in cells; merge, GFP and mFcγRIIB expression in cells. The experiments were repeated 3 times. (B) The protein expression levels of mFcγRIIB in human were examined by western blot analysis, with β-actin as a loading control. Lane 1, Control SLE group; lane 2, virus-infected SLE group; lane 3, healthy control group; lane 4, virus-infected healthy group. *P<0.05. Data are presented as the means ± SD of 3 independent experiments. mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB.

0.5% eosin (cat. no. G1120; Solarbio Life Science Co., Ltd.) for 2 min at room temperature, dehydrated in 100% ethanol for 1 min and treated with xylene for 3 min. Finally, neutral gum was used to seal the film.

The urine albumin (mouse albumin ELISA kit; cat. no. AKRAL-121; Shibayagi), serum anti-dsDNA antibody [mouse anti-double stranded DNA antibody (IgG) ELISA kit; cat. no. CSB-E11194m; Cusabio] and serum anti-nuclear antibody [moue anti-nuclear antibody (IgM) ELISA kit; cat. no. 88-50470-22; eBioscience] were detected according to the manufacturer's instructions. Serum-free FcγRIIB was measured using rabbit anti-mouse FcγRIIB monoclonal antibody (cat. no. sc-166711; 1:2,000, Santa Cruz Biotechnology, Inc.) and goat anti-mouse FcγRIIB polyclonal antibody (cat. no. sc-12815; 1:2,000; Santa Cruz Biotechnology, Inc.). Serum FcγRIIB-IgG was assayed using rabbit anti-mouse FcγRIIB monoclonal antibody (cat. no. sc-365864; 1:2,000; Santa Cruz Biotechnology, Inc.) and HRP-labeled goat anti-mouse IgG antibody (cat. no. bs-0296G-HRP; 1:5,000; Beijing Bioss Biotechnology Co., Ltd.). Lymphocytes were isolated from spleens using lymphocyte separation solution (Tianjin Hao Yang Hua Ke Co., Ltd.). B cells were sorted using

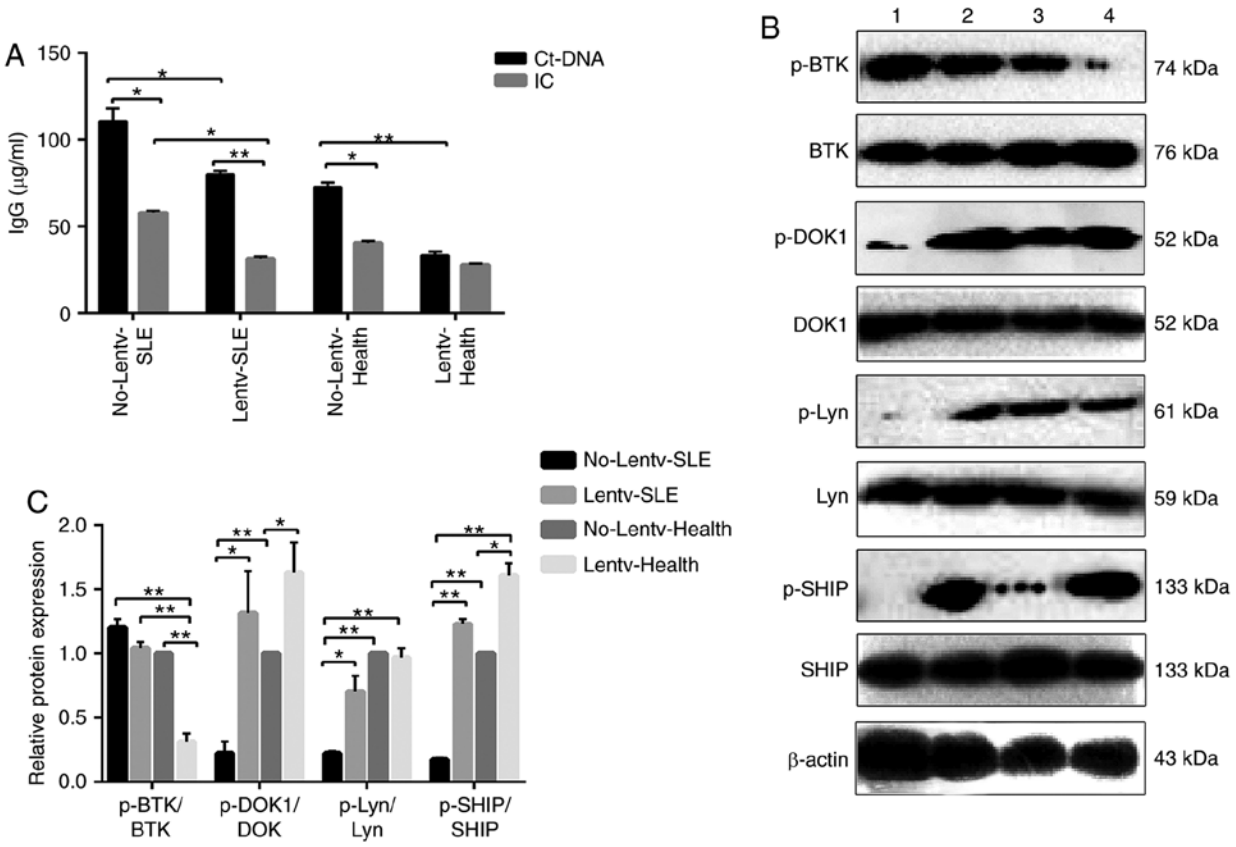

Figure 3. (A) ELISA detection of human IgG antibody levels. No-Lentv-SLE, control SLE grou; Lentv-SLE, virus-infected SLE group; No-Lentv-Health, healthy control group; Lentv-Health, virus-infected healthy group; CtDNA, calf thymus DNA. IC, anti-calf thymus DNA-immune complexes. *P<0.05; **P<0.01. The experiments were repeated 3 times. (B and C) The human protein expression levels of BTK, p-BTK, DOK1, p-DOK1, Lyn, p-Lyn, SHIP and p-SHIP were examined by western blot analysis, with β-actin as a loading control. The phosphorylation and total protein levels of BTK, DOK1, Lyn and SHIP were detected in B cells from patients with SLE and healthy subjects infected with mFcγRIIB lentivirus following anti-ctDNA-IC stimulation, and the relative level for each phosphorylation-protein/each respective total protein was calculated. No-Lentv-SLE, control SLE group; Lentv-SLE, virus-infected SLE group; No-Lentv-Health, healthy control group; Lentv-Health, virus-infected healthy group. *P<0.05; **P<0.01. Data are presented as the means ± SD of 3 independent experiments. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB; BTK, Bruton's tyrosine kinase; Lyn, Lyn proto-oncogene, Src family tyrosine kinase; DOK-1, docking protein 1; SHIP, inositol polyphosphate-5-phosphatase D.

mouse anti-CD19 microbeads (Miltenyi Biotec GmbH). The protein and phosphorylation levels of BTK, Lyn and SHIP in the B cells were detected by western blot analysis using BTK polyclonal antibody (cat. no. 21581-1-AP; 1:1,000; Proteintech), phospho-BTK antibody (cat. no. 5082T; 1:1,000; Cell Signaling Technology, Inc.), Lyn polyclonal antibody (cat. no. BS6657; 1:1,000; Bioworld), phospho-Lyn (cat. no. BS64043; 1:500; Bioworld), SHIP polyclonal antibody (cat. no. BS91238; 1:1,000; Bioworld), phospho-SHIP (cat. no. BS94059; 1:1,000; Bioworld).

Statistical analysis. Statistical analyses were performed using SPSS 23.0 (IBM Corp.) and the results are presented as the means ± standard error of mean (SEM). When the data exhibited a normal distribution, one-way analysis of variance (ANOVA) with Tukey's post hoc test were used for multiple comparisons, and the SNK-q test was used for the comparison between 2 groups. When the data exhibited a non-normal distribution, the Kruskal-Wallis test (Mann-Whitney U with Bonferroni's correction applied) was used. Statistically significant values were indicated by P<0.05. Graph construction was performed using GraphPad Prism software version 5 (GraphPad Software).

Results

Successful construction of mFcγRIIB lentivirus and mFcγRIIB expression in HT-1080 cells. The human TRE-mFcγRIIB lentiviral vector was successfully constructed with the sequencing results indicating that the inserted mFcγRIIB gene was 100% homologous to that provided by GenBank. The lentiviral vector titer was 10^6 TU/ml and the lentiviral regulatory plasmid titer was 10^5 TU/ml and exhibited good infectivity (data not shown). After infecting the HT-1080 cells with the virus, mFcγRIIB expression was induced with 1,000 ng/ml Dox. Immunofluorescence staining revealed that mFcγRIIB lentivirus successfully infected HT-1080 cells indicated by the green fluorescence emitted by expressed GFP, and mFcγRIIB was expressed in the cytomembrane of the HT-1080 cells indicated by red fluorescence (Fig. 1A). Western blot assays revealed that the mFcγRIIB expression level was positively associated with the inducer concentration, as shown in Fig. 1B.

Expression of mFcγRIIB in human B cells. Similarly, as shown in Fig. 2A, green fluorescence indicated that the mFcγRIIB lentivirus was successfully infected into B cells, and red fluorescence indicated that mFcγRIIB was expressed

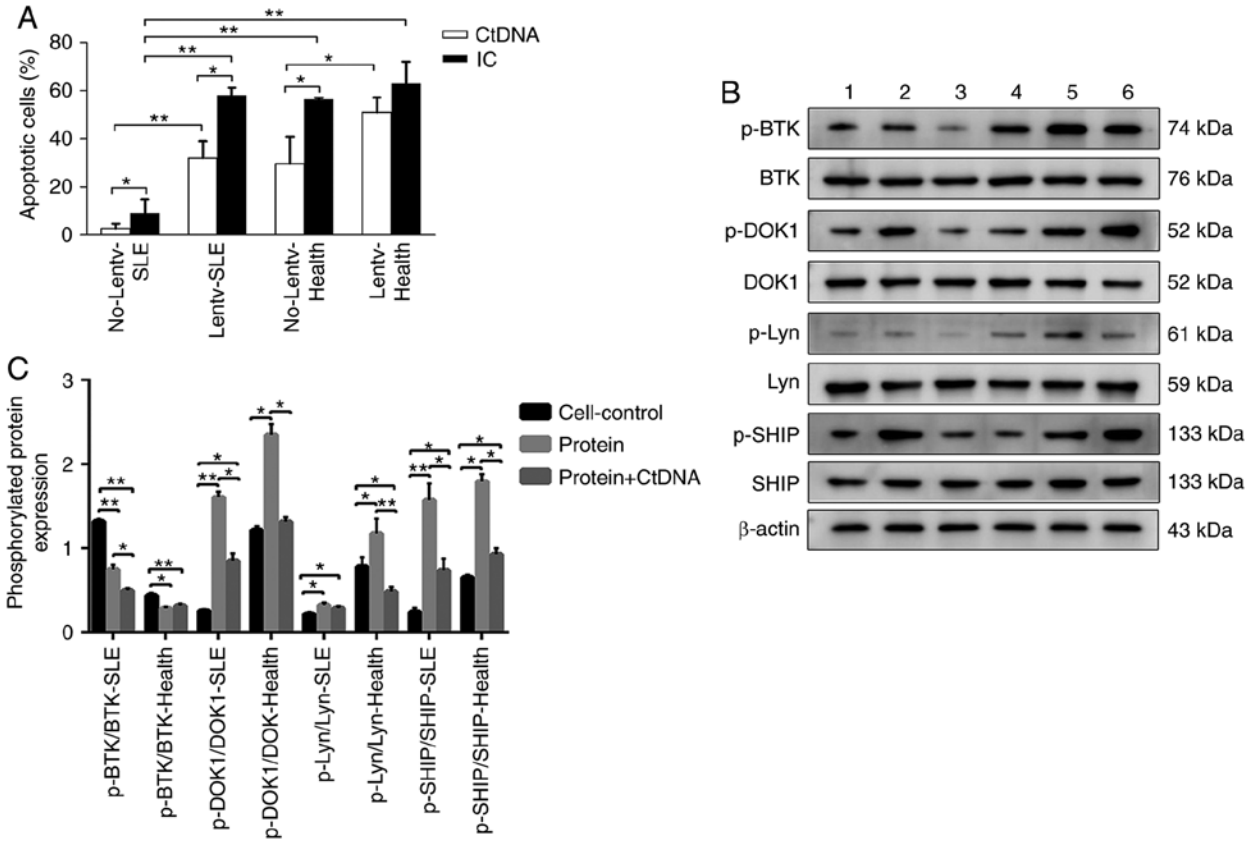

Figure 4. (A) Apoptotic rate of B cells infected by human mFcγRIIB lentivirus. No-Lentv-SLE, control SLE group; Lentv-SLE, virus-infected SLE group; No-Lentv-Health, healthy control group; Lentv-Health, virus-infected healthy group; CtDNA, calf thymus DNA; IC, anti-calf thymus DNA-immune complexes. *P<0.05; **P<0.01. The experiments were repeated 3 times. (B and C) The human protein expression levels of BTK, p-BTK, DOK1, p-DOK-1, Lyn, p-Lyn, SHIP and p-SHIP were examined by western blot analysis, with β-actin as a loading control. The phosphorylation and total protein levels of BTK, DOK-1, Lyn and SHIP were detected in B cells of patients with SLE and healthy subjects following treatment with human sFcγRIIB or sFcγRIIB plus ctDNA, and the relative level for each phosphorylation-protein/each respective total protein was calculated. Cell-Control-SLE, B cells of SLE patients; Protein-SLE, B cells of SLE patients treated with sFcγRIIB; Protein + CtDNA-SLE, B cells of SLE patients treated with sFcγRIIB and ctDNA; Cell-Control-Health, B cells of healthy subjects; Protein-Health, B cells of healthy persons treated with sFcγRIIB; Protein + CtDNA-Health, B cells of healthy persons treated with sFcγRIIB and ctDNA. *P<0.05; **P<0.01. Data are presented as the means ± SD of 3 independent experiments. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB; BTK, Bruton's tyrosine kinase; Lyn, Lyn proto-oncogene, Src family tyrosine kinase; DOK-1, docking protein 1; SHIP, inositol polyphosphate-5-phosphatase D.

Table I. Total sFcγRIIB and sFcγRIIB-IgG levels in human serum (n=30, ng/ml, means ± SD).

Group	Content
SLE total sFcγRIIB	125.11±4.63[a]
Health total sFcγRIIB	134.30±5.89
SLE sFcγRIIB-IgG	89.23±13.07[b]
Health sFcγRIIB-IgG	132.64±6.20

[a]P<0.05 vs. healthy total sFcγRIIB; [b]P<0.01 vs. healthy sFcγRIIB-IgG. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB.

in the cytomembrane of B cells. In addition, immunofluorescence staining (Fig. 2A) and western blot analysis (Fig. 2B) revealed that the expression level of endogenous mFcγRIIB in the B cells of patients with SLE was lower than that in the healthy controls. mFcγRIIB expression in the virus-infected SLE group was significantly higher than that in the control SLE group. Similarly, the expression of mFcγRIIB in the virus-infected healthy group was higher than that in the control healthy group.

Effects of overexpression of mFcγRIIB on human B cells. ctDNA and anti-ctDNA-IC were used to stimulate infected B cells. ELISA was performed to detect anti-IgG antibody secreted by B cells, and the protein and phosphorylation levels of BTK, Lyn, DOK1 and SHIP were detected by western blot analysis. The results revealed that the IgG levels in the virus-infected SLE and healthy groups were respectively significantly lower than those in the control SLE and control healthy groups. Anti-ctDNA-IC treatment significantly downregulated the B cell secretion of IgG antibody, apart from the virus-infected healthy group (Fig. 3A). The relative expression levels of p-DOK1/DOK1, p-SHIP/SHIP in the virus-infected SLE and virus-infected healthy groups were respectively significantly higher than those in the control SLE and control healthy groups; the relative expression levels of p-Lyn/Lyn in the virus-infected SLE, virus-infected healthy groups and control healthy groups were respectively significantly higher than those in the control SLE

Table II. Soluble FcγRIIB binding to immune complexes, with OD values (n=30, means ± SD).

Group	OD value						
DNA (mg/ml)	0	0.05	0.1	0.2	0.4	0.8	1.0
SLE	1.239±0.061[a]	1.439±0.065[a]	1.507±0.065[a]	1.569±0.068[a]	1.653±0.075[a]	1.763±0.068[a]	1.776±0.065[a]
Healthy	1.776±0.065	1.258±0.054	1.323±0.063	1.394±0.065	1.464±0.072	1.546±0.072	1.557±0.067
PBS	0.097	0.076	0.083	0.086	0.091	0.072	0.095

[a]P<0.05 vs. healthy group. SLE, systemic lupus erythematosus; sFcγRIIB, soluble FcγRIIB.

Table III. IgG levels in B cells (n=30, μg/ml, means ± SD).

Group	IgG concentration
Control-Health	19.608±4.838
sFcγRIIB-Health	6.054±1.656[a]
sFcγRIIB-ctDNA-Health	12.400±1.803[a,b]
Control-SLE	113.389±6.768
sFcγRIIB-SLE	33.392±1.521[c]
sFcγRIIB-ctDNA-SLE	70.679±6.595[d,e]

[a]P<0.01 vs. Control-Healthy group; [b]P<0.05 vs. sFcγRIIB-Healthy group; [c]P<0.01 vs. Control-SLE group; [d]P<0.05 vs. Control-SLE group; [e]P<0.05 vs. sFcγRIIB-SLE group. SLE, systemic lupus erythematosus; sFcγRIIB, soluble FcγRIIB.

group, while the relative expression of p-BTK/BTK in the virus-infected SLE and virus-infected healthy groups was significantly lower than that in the control SLE and control healthy groups following anti-ctDNA-IC stimulation (Fig. 3B and C). More importantly, the apoptotic rate of the B cells in the virus-infected SLE and healthy groups was respectively significantly higher than that in the control SLE and control healthy groups, and the apoptotic rate of the B cells in the anti-ctDNA-IC treatment group was higher than that in the ctDNA treatment group (Fig. 4A).

sFcγRIIB and sFcγRIIB-IgG levels in serum of patients with SLE. The levels of sFcγRIIB and sFcγRIIB-IgG in the serum of patients with SLE were lower than those in the serum of the healthy subjects. The level of sFcγRIIB-IgG in the patients with SLE was lower than that in the healthy subjects. No significant difference was observed between sFcγRIIB-IgG and sFcγRIIB in the serum of the healthy subjects (Table I).

Effect of sFcγRIIB on human B cells. Recombinant sFcγRIIB protein was successfully expressed and identified by western blot analysis. The results revealed that recombinant sFcγRIIB bound to immune complexes (ICs) in serum and reduced the secretion of IgG antibodies in B cells, suggesting that sFcγRIIB may inhibit the activation of B lymphocytes by combining with IC (Tables II and III). The relative expression levels of p-Lyn/Lyn p-DOK1/DOK1 and p-SHIP/SHIP in the SLE groups were lower, while the relative expression of p-BTK/BTK was higher compared to the corresponding healthy groups. Furthermore, among the SLE groups, the relative expression levels of p-Lyn/Lyn, p-DOK1/DOK1 and p-SHIP/SHIP in the sFcγRIIB treatment subgroups were higher, while the relative expression of p-BTK/BTK was lower than that in the control subgroup (Fig. 4B and C).

Effect of mFcγRIIB lentivirus on MRL/lpr SLE mice. The mouse mFcγRIIB gene was successfully inserted into the TRE vector and packed into viruses. The titers of the expressed and regulated viruses were measured to reach 10^6 TU/ml. mFcγRIIB lentivirus and lentivirus regulatory plasmid were injected into the MRL/lpr SLE mice, and the effects were examined. As a result, in the prevention group and treatment group, mFcγRIIB lentivirus treatments significantly decreased urine albumin, serum anti-dsDNA antibody and serum anti-nuclear antibody levels, while increasing mFcγRIIB expression in spleen B cells (Fig. 5). In the prevention and treatment groups of B cells, the relative expression of p-BTK/BTK was increased, while the relative expression of p-SHIP/SHIP was decreased (Fig. 6). More importantly, the results of H&E staining revealed that in the MRL/lpr SLE mouse kidney tissue, a large number of lymphocytes infiltrated around the glomerulus and the glomerular capillaries became atrophied and hardened. In the liver tissue, mild histological alterations were observed, such as ballooning in the cytoplasm, dilatation in the central vein and hepatic sinusoids, and lymphocytic infiltrate in the hepatic lobules. In the lymph nodes, there were a large number of lymphocytes in the lymphoid follicles, the lymphoid nodules were enlarged, and the lymphoid follicles were proliferated. However, mFcγRIIB lentivirus treatments slightly ameliorated these pathologies, including decreased cytoplasmic swelling and lymphocyte infiltration (Fig. 7).

Preventive and therapeutic effects of sFcγRIIB in MRL/lpr SLE mice. Mouse sFcγRIIB gene was successfully cloned into the pET-sFcγRIIB plasmid, which was identified by DNA sequencing, and mouse recombinant sFcγRIIB protein was obtained by induction and was purified. Following treatment with mouse recombinant sFcγRIIB protein, some indexes in the MRL/lpr SLE mice were detected. As a result, in the prevention and treatment groups, in all sFcγRIIB protein treatments, the levels of sFcγRIIB and sFcγRIIB-IgG were significantly increased in a positive dose-dependent manner, while serum

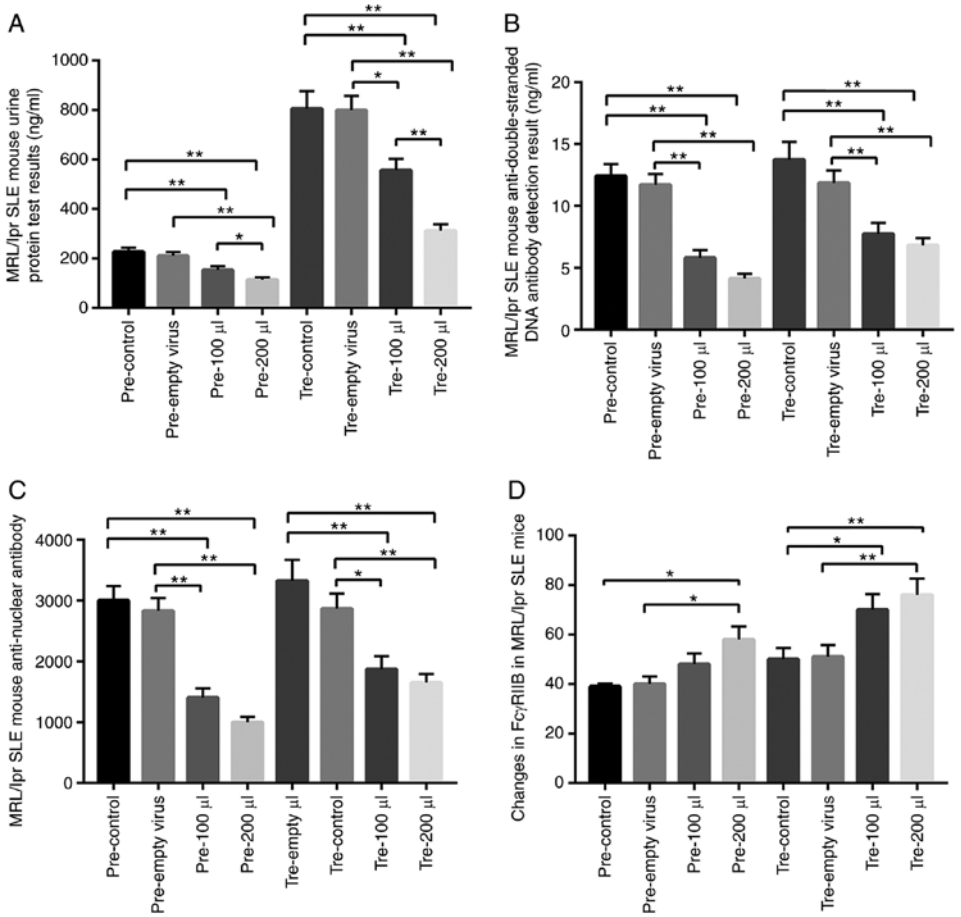

Figure 5. Target detection of MRL/lpr SLE mice following mFcγRIIB treatment. (A) Changes in urine protein levels. (B) Changes in serum anti-dsDNA antibody levels. (C) Changes in serum anti-nuclear antibody levels. (D) Changes in mFcγRIIB levels in B cells. pre-control, Control subgroup in the prevention group; pre-empty virus, empty virus subgroup in the prevention group; pre-100 μl, 100 μl mFcγRIIB lentivirus subgroup in the prevention group; pre-200 μl, 200 μl mFcγRIIB lentivirus subgroup in the prevention group; tre-control, control subgroup in the treatment group; tre-empty virus, empty virus subgroup in the treatment group; tre-100 μl, 100 μl mFcγRIIB lentivirus subgroup in the treatment group; tre-200 μl, 200 μl mFcγRIIB lentivirus subgroup in the treatment group. *P<0.05; **P<0.01; n=10 in each group. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB.

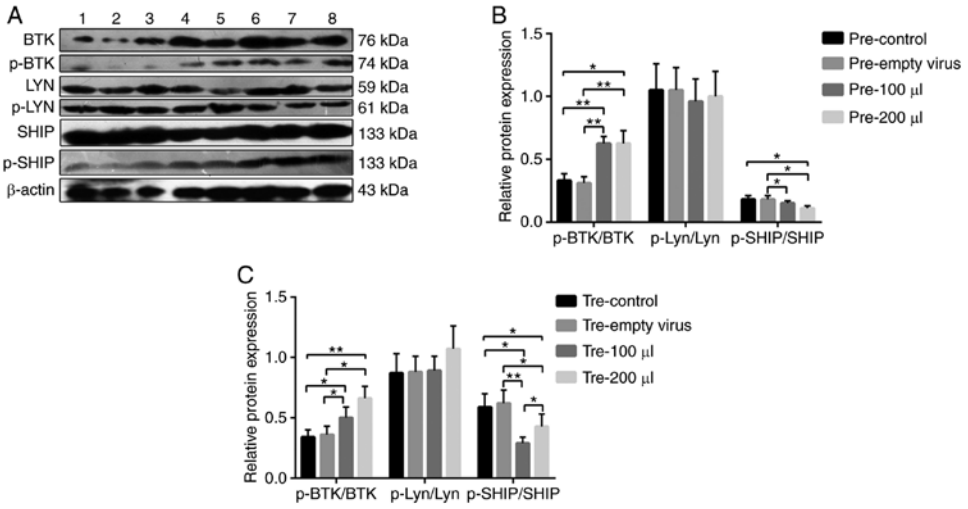

Figure 6. (A) The protein expression levels of BTK, p-BTK, Lyn, p-Lyn, SHIP and p-SHIP in MRL/lpr SLE mouse B cells were examined by western blot analysis, with β-actin as a loading control. (B and C) The phosphorylation and total protein levels of BTK, Lyn and SHIP were detected in B cells from MRL/lpr SLE mice after the infection of mFcγRIIB lentivirus, and the relative level for each phosphorylation-protein/each respective total protein was calculated. Lane 1: pre-empty virus, empty virus subgroup in the prevention group; lane 2: pre-control, control subgroup in the prevention group; lane 3: pre-100 μl, 100 μl mFcγRIIB lentivirus subgroup in the prevention group; lane 4: pre-200 μl, 200 μl mFcγRIIB lentivirus subgroup in the prevention group; lane 5: tre-control, control subgroup in the treatment group; lane 6: tre-empty virus, empty virus subgroup in the treatment group; lane 7: tre-100 μl, 100 μl mFcγRIIB lentivirus subgroup in the treatment group; lane 8: tre-200 μl, 200 μl mFcγRIIB lentivirus subgroup in the treatment group. *P<0.05; **P<0.01. Data are presented as means ± SD of 3 independent experiments. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB; BTK, Bruton's tyrosine kinase; Lyn, Lyn proto-oncogene, Src family tyrosine kinase; DOK-1, docking protein 1; SHIP, inositol polyphosphate-5-phosphatase D.

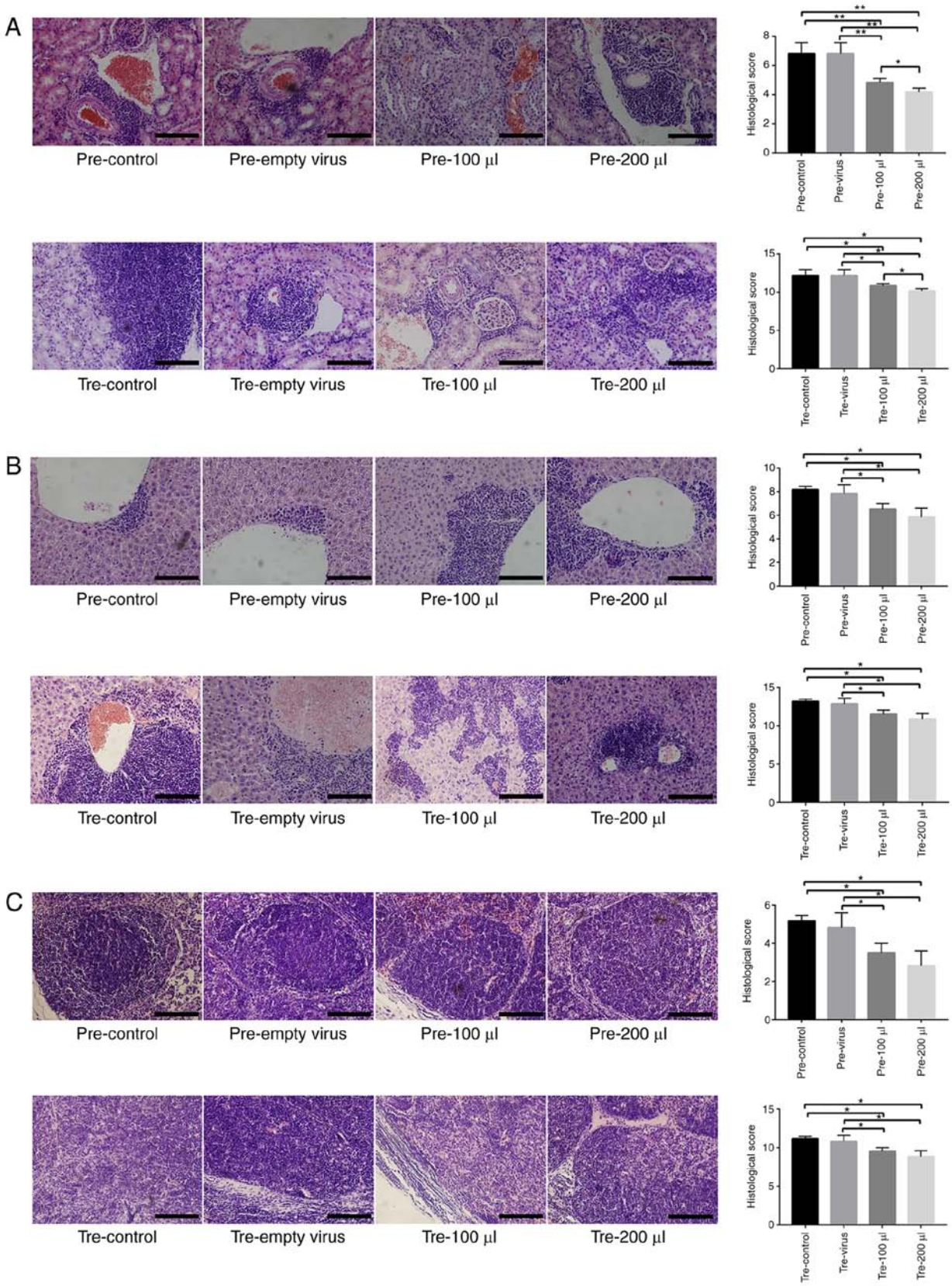

Figure 7. H&E staining results in mice. (A) Kidney tissue. (B) Liver tissue. (C) lymph gland. Representative images for HE results are shown. Scale bar, 25 μm. *P<0.05; **P<0.01; n=10 in each group.

anti-dsDNA antibody and urine albumin levels were markedly decreased in a reverse dose-dependent manner (Fig. 8), as compared to the normal saline treatment. Moreover, the relative expression levels of p-Lyn/Lyn and p-SHIP/SHIP were increased, while the relative expression of p-BTK/BTK in B cells was decreased following sFcγRIIB protein treatment (Fig. 9).

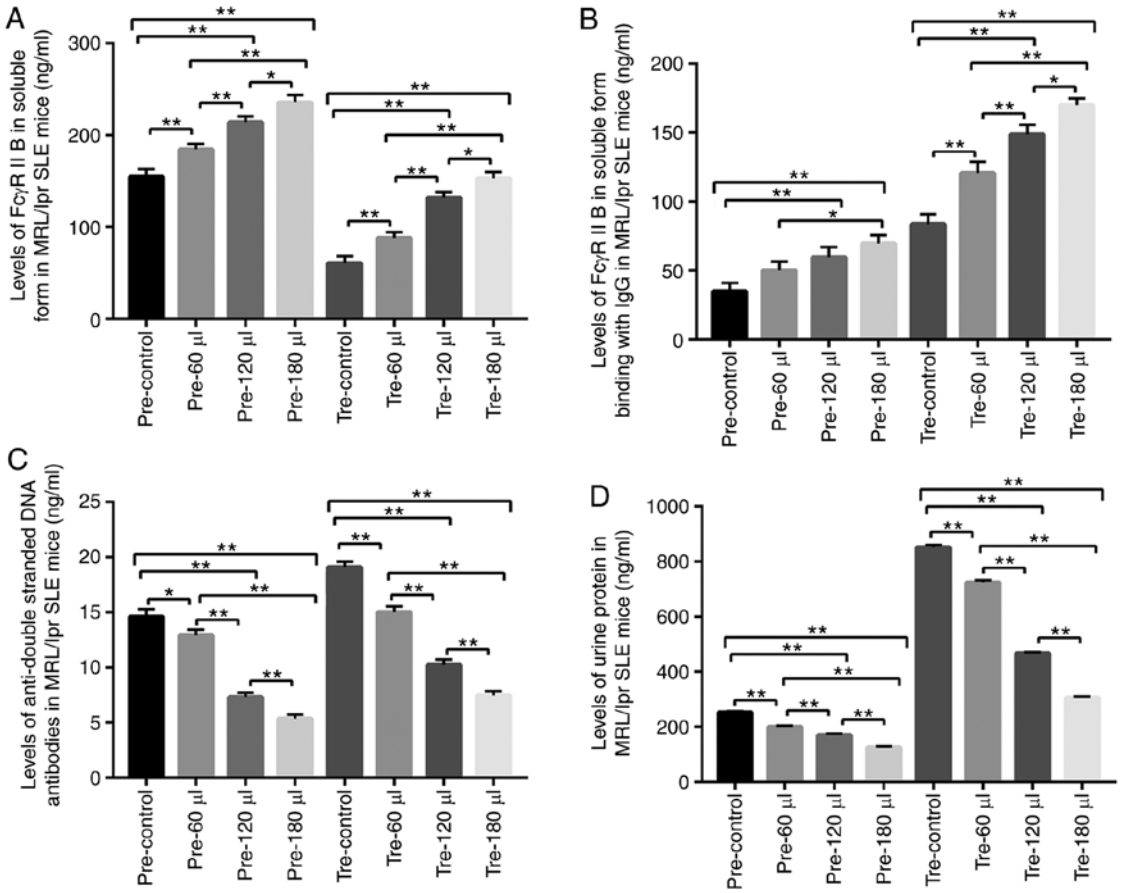

Figure 8. Target detection in MRL/lpr SLE mice. (A) Detection of sFcγRIIB. (B) Detection of FcγRIIB binding with IgG. (C) Detection of serum anti-double-stranded DNA antibody. (D) Detection of urine protein. pre-control, control subgroup in the prevention group; pre-60 μl, 4.8 μg sFcγRIIB subgroup in the prevention group; pre-120 μl, 9.6 μg sFcγRIIB subgroup in the prevention group; pre-180 μl, 14.4 μg sFcγRIIB subgroup in prevention group; tre-control, control subgroup in the treatment group; tre-60 μl, 4.8 μg sFcγRIIB subgroup in the treatment group; tre-120 μl, 9.6 μg sFcγRIIB subgroup in the treatment group; tre-180 μl, 14.4 μg sFcγRIIB subgroup in the treatment group. *P<0.05; **P<0.01; n=10 in each group. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB.

Discussion

FcγRIIB, an inhibitory receptor in the FcγR family (18), is composed of an extracellular domain containing two Ig-like structures and an intracellular region containing an ITIM motif (19,20). The extracellular region is an Fc region with a low affinity for binding all IgG subclasses and exists as a membrane-bound type and insoluble form. sFcγRIIB is produced by the hydrolysis of the mFcγRIIB extracellular region stimulated by antigens and cytokines or is produced by FcγRIIB gene splicing (21,22). In the present study, in order to examine the roles of mFcγRIIB and sFcγRIIB in SLE, patients with SLE and MRL/lpr SLE mouse models were utilized.

The serum of patients with SLE contains a large number of anti-nuclear antibodies and IgG type CICs (23), and research has indicated that FcγRIIB can be crosslinked to BCR or activated FcR by IC to inhibit the activation and antibody secretion of B cells (24,25). Therefore, in the present study, ctDNA and anti-ctDNA-IC were used to stimulate B lymphocytes from patients with SLE and healthy subjects following mFcγRIIB lentivirus transfection. It was found that ctDNA stimulation reduced B lymphocyte IgG antibody secretion following mFcγRIIB lentivirus transfection, and exerted a more prominent effect on SLE B cells than on normal B cells. Furthermore, when the added IC reached a certain level, it directly inhibited the secretion of IgG antibody by B cells. Therefore, B cell-derived FcγRIIB is involved in preventing the *in vivo* production of autoreactive antibodies, providing a peripheral checkpoint for maintaining normal self-tolerance. The combination of FcγRIIB and IC provides an inhibitory feedback loop for B lymphocytes, thus maintaining the dynamic balance of antibody expression.

Following lentivirus vector transfection into MRr/lpr mice, the expression level of mFcγRIIB was increased in a dose-dependent manner with the lentiviral vector. In the presence of the DOX inducer, the transcriptional expression of the target gene was induced by the combining of expression product of regulatory plasmid with rtTA of the response plasmid. Therefore, the concentration of DOX inducer directly affects the transcription and expression levels of target genes (26). The levels of anti-nuclear antibodies, urinary albumin and anti-dsDNA in MRr/lpr mice were downregulated after the injection of virus containing Dox, due to the increase in mFcγRIIB which decreased the B cell secretion of antibodies. These results could deactivate signal transduction proteins by dephosphorylation to terminate BCR activation

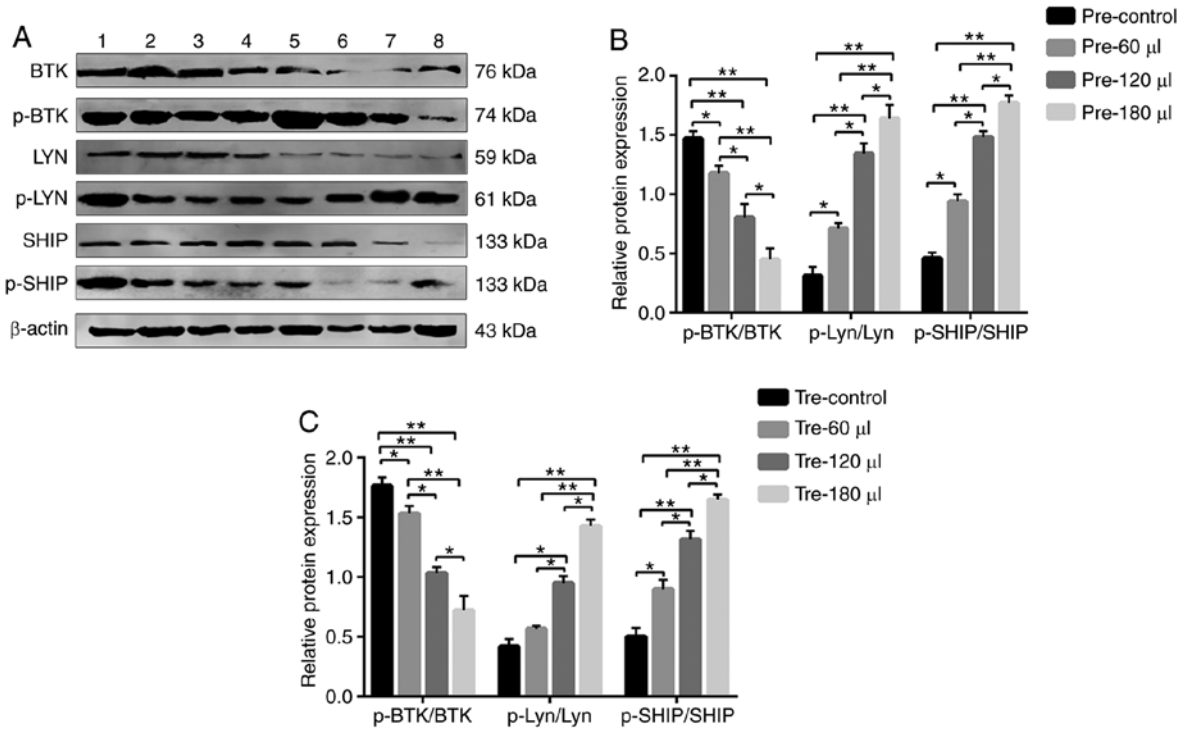

Figure 9. (A) The protein expression levels of BTK, p-BTK, Lyn, p-Lyn, SHIP and p-SHIP in MRL/lpr SLE mouse B cells were examined by western blot analysis, with β-actin as a loading control. (B and C) The phosphorylation and total protein levels of BTK, Lyn and SHIP were detected in B cells from MRL/lpr SLE mice after the infection of sFcγRIIB lentivirus, and the relative level for each phosphorylation-protein/each respective total protein was calculated. Lane 1: pre-control, control subgroup in the prevention group; lane 2: pre-60 μl, 4.8 μg sFcγRIIB subgroup in the prevention group; lane 3: pre-120 μl, 9.6 μg sFcγRIIB subgroup in the prevention group; lane 4: pre-180 μl, 14.4 μg sFcγRIIB subgroup in the prevention group; lane 5: tre-control, control subgroup in the treatment group; lane 6: tre-60 μl, 4.8 μg sFcγRIIB subgroup in the treatment group; lane 7: tre-120 μl, 9.6 μg sFcγRIIB subgroup in the treatment group; lane 8: tre-180 μl, 14.4 μg sFcγRIIB subgroup in the treatment group. *P<0.05; **P<0.01; n=10 in each group. SLE, systemic lupus erythematosus; mFcγRIIB, membrane-bound type FcγRIIB; sFcγRIIB, soluble FcγRIIB; BTK, Bruton's tyrosine kinase; Lyn, Lyn proto-oncogene, Src family tyrosine kinase; DOK-1, docking protein 1; SHIP, inositol polyphosphate-5-phosphatase D.

signal transduction, inhibiting the activation, proliferation, differentiation and antibody production of B cells (27).

The FcγRIIB signaling pathway is closely related to IgG antibody secretion in B cells. It mainly inhibits cell activation and proliferation through two signaling pathways (28). Pauls and Marshall (29) and Wang *et al* (30) found that FcγRIIB activated tyrosine kinase (Lyn) by crosslinking with BCR through IC, causing the tyrosine phosphorylation of ITIM to recruit molecules, such as SHIP (inositol phosphatase). SHIP acts on 3,4,5-phosphatidylinositol triphosphate to hydrolyze it to 3,4-phosphatidylinositol diphosphate. 3,4-Phosphatidylinositol diphosphate prevents PIP3 from recruiting BTK and phospholipase cγ2 (PLCγ2) onto cell membranes following dephosphorylation, thereby reducing the intracellular calcium levels, which finally inhibits cell activation involving related kinases to reduce antibody production. ITIM transduction can inhibit cell proliferation by the activation of DOK1 and MAP kinase instead of SHIP (29,30). High concentrations of FcγRIIB lead to the direct apoptosis of B cells by crosslinking with BCR to increase the phosphorylation of Lyn and SHIP1, and the simultaneous phosphorylation of BTK, independent of the ITIM pathway.

Corneth *et al* found that the cytoplasmic regions of human and mouse FcγRIIB proteins contained ITIM motifs (28). This highly conserved 13 amino acid sequence encoded by the C3 exon contains a common inhibitory sequence, I/VxYxL, which can prevent the ITIM motif from transmitting activation signaling by cross-linking BCR, FcγRI, and FcγRIII. When FcγRIIB crosslinks other activation receptors containing ITIM, ITIM can inhibit cell activation and proliferation by activating Lyn to recruit SHIP1 or by activating DOK1 without SHIP recruitment (28).

It has been reported that recombinant human sFcγRIIB inhibited SLE membrane-type IC-mediated tissue damage by interfering with the *in vitro* binding of IC to B cells (31,32). Recombinant human sFcγRIIB plays a role by competitively binding IgG sites with mFcγRIIB *in vivo*; however, the mechanisms responsible for this blocking effect have not been extensively investigated (33).

Recombinant human sFcγRIIB inhibits the *in vitro* secretion of IgG-type antibodies by B cells. However, the mechanisms involved remain unclear. In the present study, following the stimulation of B cells from patients with SLE with recombinant human sFcγRIIB to secrete IgG-type antibodies, BTK, Lyn, SHIP and DOK1 were detected downstream of the sFcγRIIB and BCR signal transduction pathways. As a result, the phosphorylation levels of Lyn, SHIP and DOK1 were increased, while the phosphorylation level of BTK was decreased, consistent with those mediated by mFcγRIIB signal transduction, which proved that recombinant human sFcγRIIB binds to mFcγRIIB by bridging IgG type anti-sFcγRIIB autoantibody, and then initiated a series of signal transduction pathways. Inhibitory signals produced by human sFcγRIIB antagonizes activation signals produced by BCR, which eventually leads to B cell inhibition.

Recombinant human sFcγRIIB has been shown to alleviate the symptoms of proteinuria and weight loss in NZB/NZW F1 SLE model mice induced by IC, and to improve the survival rate of model mice with prophylactic application (34). However, this previous study did not explore the detailed mechanisms of sFcγRIIB alleviating SLE. After injecting human sFcγRIIB into each group of model mice, it was observed that the serum sFcγRIIB levels in the prevention and treatment groups increased with the increased dose of injected sFcγRIIB, and the sFcγRIIB level in the prevention group was higher than that in the treatment group. Simultaneously, the titers of anti-dsDNA antibodies and the urinary protein in the 2 groups decreased. Recombinant human sFcγRIIB inhibited the *in vivo* B cell secretion of IgG antibody. Of note, it was found in the present study that the phosphorylation levels of Lyn and SHIP were increased significantly with an increase in the sFcγRIIB dose injected into SLE model mice, which may have inhibited the downstream function of ITIM signal regulation and the release of calcium ions through the BCR signal transduction pathway (35). The phosphorylation levels of BTK in the prevention and treatment groups were significantly decreased with the increase in exogenous sFcγRIIB, indicating that the phosphorylation of BTK was not blocked when sFcγRIIB was deficient, while the phosphorylation of BTK was inhibited when sFcγRIIB was restored or enhanced. Therefore, it was surmised that sFcγRIIB inhibited the activation of B cells by inhibiting the activation of BCR pathway.

In conclusion, the present study demonstrated that recombinant FcγRIIB inhibited B cell IgG antibody secretion by altering the phosphorylation levels of downstream proteins involved in the FcγRIIB signaling pathway. Simultaneously, the transduction of mFcγRIIB lentivirus into MRr/lpr SLE mice further improved the symptoms of SLE mice, suggesting that FcγRIIB may play a role in improving the activation, proliferation and antibody secretion of B cells, providing a new approach and method for the prevention and treatment of SLE patients. However, further experiments are required to address the conclusion on how FcγRIIB regulates the phosphorylation of downstream proteins of FcγRIIB signaling pathway, suppresses B cell activation to ameliorate systemic lupus erythematosus, and promotes the apoptosis of B cells.

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Availability of data and materials

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

Authors' contributions

LS and XC performed the experiments, analyzed the data and wrote the manuscript. SC, JW, HX and HL performed the experiments and collected the data. ZY conceived and designed the study, obtained the funding and revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved the Ethics Committee of Ningxia Medical University. All subjects signed informed consent forms prior to their inclusion in the study. The animal experiments were approved by the Animal Ethics Committee of Ningxia Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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