Research Article

Stabilized β-Catenin Ameliorates ALPS-Like Symptoms of B6/lpr Mice

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1. Background

To maintain homeostasis, apoptosis plays a critical role in lymphocyte development and activity. Excessive lymphocyte apoptosis can cause immune defects; however, inhibition of normal apoptosis can lead to the autoimmune disease or the occurrence of lymphoma [1]. So the immune system has developed some apoptotic mechanisms, in which activation-induced cell death (AICD) can be used to inhibit the excessive proliferation of T lymphocytes [1, 2]. Previous study reported that the proteins encoded by Fas and FasL genes were involved in the apoptosis of lymphocytes [3, 4]. As a major suppressing regulation, the AICD controls the homeostasis of T cell during immune response, in which activated T lymphocytes upregulate the expression of Fas (CD95), then interact with the Fas Ligand (FasL, CD95L) on activated B and T lymphocyte surface through the Fas-activating death domain (FADD) to trigger the caspase cascade, leading to proteolysis, DNA degradation, and induction of cell apoptosis [5].

β-Catenin, encoded by the CTNNB1 gene, is a multifunctional protein. It has dual roles in the cell, one of which is as the most important information molecule in the canonical Wnt pathway to regulate cell growth, differentiation, and apoptosis [6]. In the absence of Wnt signal stimulation, the target genes are usually kept inactive and bound by transcriptional corepressors. A cytosolic β-catenin “destruction” complex is composed of the adenomatous polyposis
coli (APC), axin, and glycogen synthase kinase-3-β (GSK3β), which marks β-catenin for ubiquitination and proteosomal degradation by phosphorylating it in the N-terminus [7]. In response to Wnt signals, binding of Wnt ligands to the frizzled receptors and the lipoprotein receptor-related protein (LRP) coreceptors on the cell surface disrupt the “destruction” complex and inactivation of GSK3β-mediated phosphorylation and degradation of β-catenin [7, 8]. As a result, nonphosphorylated β-catenin is accumulated in the cytoplasm, translocates into the nucleus, displaces the Grg/Tle corepressors, and together with T cell factors leads to the activation of Wnt target genes.

Autoimmune lymphoproliferative syndrome (ALPS) is caused by the defect of Fas-mediated apoptosis and characterized by nonmalignant autoimmunity lymphoproliferation with massive accumulation of lymphocytes and resulting in splenomegaly and lymphoadenopathy [9, 10]. The mutations characterized by nonmalignant autoimmunity lymphoproliferation caused by the defect of Fas-mediated apoptosis and characterized in previous study [19]. C57BL/6 background mice of China (Nanjing, Jiangsu, China) and crossed with β-cat<sup>fl</sup> mice to obtain β-cat<sup>fl</sup> lpr/lpr mice at age 2 months, and the genotypes were identified and displayed in Figure 1. C57BL/6 WT (wild type) mice were purchased from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China). In the present study, all used mice were maintained at the specific pathogen-free Animal Research Center under a 12-hour light/12-hour dark cycle at 22°C in Sun Yat-sen University Laboratory Animal Center (Guangzhou, China) and used at age 6 months according to the Institutional Animal Care and Use Committee. All female mice were divided into WT, lpr/lpr, β-cat<sup>fl</sup>, and β-cat<sup>fl</sup> lpr/lpr groups, each group with 6 to 10 in each experiment.

2.2. The Genotype Identification of Offspring Mice. When the pups were 4 weeks, the tail DNA was extracted and used for genotyping. Briefly, 1 cm tail was digested overnight at 55°C in 0.5 ml reagent containing 200 μg/ml proteinase K. Then the DNA samples were extracted according to the conventional protocol, including 500 μl phenol chloroform extract, 1:1 isopropyl alcohol precipitation, 500 μl (75%) ethanol washing, and 100 μl ddH<sub>2</sub>O dissolving. PCR reaction was performed using the Ready Mix Taq solution (Takara, Beijing, China) with an Applied Biosystems (2720 Thermal Cycler, USA). β-cat<sup>fl</sup> genotyping including β-catenin-Tg-K1 and β-catenin-Tg-R-K2 two primers, and Fas genotyping including Fas-C, Fas-W, and Fas-K three primers (Table S1 available online at https://doi.org/10.1155/2017/3469108). β-cat<sup>fl</sup> and Fas genotyping were, respectively, subjected to the following conditions: 994°C 3 min, (94°C 30 s, 60°C 30 s, and 72°C 30 s) for 35 cycles, and 72°C 5 min; 94°C 3 min, (94°C 30 s, 59°C 1 min and 72°C 30 s) for 35 cycles, and 72°C 5 min. The PCR products were separated by electrophoresis using 2% and 3% agarose (BIOWEST™, Chai Wan, Hong Kong) with Gene Ruler™ 1 kb Plus (Fermentas, Shenzhen, China).

2.3. Cell Activation and Apoptosis In Vitro. Splenocytes were isolated from 8- to 12-week-old mice. CD4<sup>+</sup> T cells were purified using a MACS magnetic column with a CD4 T cell enrichment kit (eBioscience, USA). The cells (1 × 10<sup>7</sup>) were cultured for 5 days in fresh medium containing IL-2 (5 ng/ml), and cells (2 × 10<sup>5</sup>) were restimulated in 96-well plates containing different concentrations of plate-bound anti-CD3. Cell cultures were supplemented with IL-2 (5 ng/ml), IL-7(100 ng/ml), and IL-15 (100 ng/ml) to prevent spontaneous apoptosis. Cells were then harvested and washed once with ice-cold PBS supplemented with 1% FCS. Cell pellets were stained with anti-CD3-APC, annexin V, and 7-AAD (BD Biosciences) following the manufacturer’s protocol. Analyses were performed on a FACS-caliber (BD Biosciences) with CELLQuest software.

2.4. Antibodies and Flow Cytometry. Anti-mouse CD3 (clone PC-61) (QuantoBio), anti-mouse TCRβ chain (clone H75-597) (eBioscience, San Diego, CA, USA), anti-mouse CD4 (clone RM4-5) (eBioscience), anti-mouse CD8α (clone 53-6.7) (eBioscience), rat anti-mouse CD44 (clone IM7) (BD Biosciences, Franklin Lakes, NJ, USA), and rat anti-mouse
CD62L (MEL-14) (BD Biosciences) fluorochrome-labeled antibodies were used for flow cytometry. Single cell suspension of spleen and peripheral blood from the mice was prepared as previously described [21] and stained with the fluorochrome-labeled antibodies, and the fluorescence intensity was analyzed using a FACS Calibur Instrument (BD Biosciences, Franklin Lakes, NJ, USA). FlowJo software version 7.2.5 (TreeStarInt, USA) was used to analyse the intensity was analyzed using a FACS Calibur Instrument (BD Biosciences, Franklin Lakes, NJ, USA). FlowJo software version 7.2.5 (TreeStarInt, USA) was used to analyse.

2.5. Serum Cytokines Analysis. To measure the serum cytokine levels of mice, including IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-17, TNF-α, IFN-γ, G-CSF, GM-CSF, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and Eotaxin, we used a Bio-Plex Pro™ Mouse Cytokine 23-Plex panel (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer’s protocols. Array analysis was performed using the Bio-Plex® Protein Array system (Bio-Rad Laboratories, Hercules, CA, USA) following manufacturer instructions.

2.6. ELISA for Serum Anti-dsDNA Antibody. Blood was collected at the age of 6 months and serum was isolated and stored at −80°C upon use. The levels of serum anti-dsDNA antibody were measured by ELISA with homemade ELISA kit. Shortly, dsDNA-coated plates pretreated with fetal bovine serum blocking (FBS; Hyclone, Logan, UT, USA) were incubated with sample serum in series dilution, then incubated with HRP-conjugated rabbit anti-mouse IgG (whole molecule) secondary antibodies (Sigma, St. Louis, MO, USA). TMB single solution (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) was used to develop the color, and the signal was detected on an automatic microplate reader (Thermo Scientific™ Multiskan™ MK3, Waltham, MA, USA) at 450 nm with a reference wavelength of 630 nm.

2.7. Histopathology. Kidneys were fixed in 4% paraformaldehyde solution overnight at room temperature and embedded in paraffin. The 4 μm tissue slides were cut and stained with hematoxylin (H&E) or periodic acid schiff (PAS) for routine histological analysis. Sections were visualized by light microscopy at 400x magnification. The H&E and PAS-stained tissue slides were scored blindly by semiquantitative system according to the guidelines previously described [22].

2.8. Statistics Analysis. All data are shown as the mean ± SD. The unpaired t-test was used to compare two independent groups by the GraphPad Prism software 5.0 (GraphPad Software, San Diego, CA, USA). Significance was achieved when p < 0.05.

3. Results

3.1. Stabilized β-Catenin Potentiated the Apoptosis of T Cells of lpr/lpr Mice. We have found that stabilized β-catenin potentiated Fas-mediated T cell apoptosis [20]. To further elucidate the role of transgenic β-catenin in the deletion of peripheral T cells, the splenic CD4+ T cells of WT, β-catenin, lpr, and β-catenin/lpr/lpr mice were challenged with anti-CD3 Ab in vitro. We found that anti-CD3 Ab stimulation significantly increased the apoptosis of CD3+ T cells in a concentration-dependent manner (Figure S1). Consistent with previous findings [20], the apoptosis of CD3+ T cells were significantly increased in β-catenin mice compared with WT mice at all concentrations of anti-CD3 Ab stimulation (Figure 2). However, in CD3+ T cells from lpr/lpr mice, the percent of apoptotic cells had no obvious difference compared with WT mice (Figure 2). Beyond that, we found that the apoptotic percent of CD3+ T cells was significantly decreased in β-catenin/lpr/lpr mice compared with β-catenin mice at all concentrations of anti-CD3 Ab stimulation but was...
higher than that in WT and lpr/lpr mice (Figure 2). These results demonstrated that β-catTg could not only potentiate Fas-mediated T cell apoptosis, but also potentiate the apoptosis of T cells in lpr/lpr mice.

3.2. Stabilized β-Catenin Slightly Ameliorated the Augments of Splenic Lymphocytes of lpr/lpr Mice. In the present study, we analyzed the splenic lymphocytes by Flow Cytometry (Figure 3(a)). We found that β-catTg decreased the numbers of T cells, CD4+ T cells, and CD8+ T cells compared with WT mice (Figure 3(b)). Meanwhile, we found that the numbers of splenocytes, T cells, and CD4+ T cells were significantly increased in lpr/lpr mice compared with WT mice, but they were slightly declined in β-catTg/lpr/lpr mice compared with lpr/lpr mice because of stabilized β-catenin, although not statistically significant (Figure 3(b)). Moreover, as the remarked change of ALPS, DNT cells were decreased in β-catTg/lpr/lpr mice compared with lpr/lpr mice, in which they were significantly increased compared with WT mice (Figure 3(b)). These data indicated that stabilized β-catenin, at some level,
Figure 3: Stabilized β-catenin slightly ameliorated the augments of splenic lymphocytes of lpr/lpr mice. All female animals were sacrificed at age 6 months, and splenocytes were isolated and stained with indicated surface markers. (a) The representative FACS results of CD4⁺ and CD8⁺ T cell subpopulation, gating on TCRβ positive cell. (b) Statistic analysis of the number of spleen cells, T cells, CD4⁺, CD8⁺, and DNT (TCRβ⁺CD4⁺CD8⁻) cells and the ratios of CD4 to CD8 in different genotype mice. Data are shown as mean ± SD (n = 6–10). *p < 0.05; **p < 0.01; and ***p < 0.001.
3.3. Stabilized β-Catenin Affected the Formation of Memory T Cells. We used surface markers CD44 and CD62L to divide spleen CD4⁺ T and CD8⁺ T subpopulations which included effector T cell (Tₑₑ), effector memory T cell (Tₑₑₘₑₑ), center memory T cell (Tₑₑₘₑₑ), and naïve T cell (Tₙₙ) (Figure 4(a)). Except for Tₑₑₘₑₑ, they displayed the same tendency between CD4⁺ and CD8⁺ T cells (Figures 4(b) and 4(c)). In both CD4 and CD8 T cells, the numbers of Tₑₑₘₑₑ were significantly increased in lpr/lpr mice compared with WT mice, then rescued by β-cat⁻fi in β-cat⁻flpr/lpr mice (Figures 4(b) and 4(c)). However, the Tₑₑ in β-cat⁻flpr/lpr mice was increased compared with lpr/lpr and WT mice (Figures 4(b) and 4(c)). In addition, either in CD4 or CD8 T cells, the numbers of Tₑₑ in β-cat⁻flpr/lpr mice and naïve T cell (TN) (Figure 4(a)). Except for Tₑₑₘₑₑ, they displayed the same tendency between CD4⁺ and CD8⁺ T cells (Figures 4(b) and 4(c)). In both CD4 and CD8 T cells, the numbers of Tₑₑₘₑₑ were dramatically decreased compared with WT mice, and β-cat⁻flpr/lpr mice also displayed a significant decline compared with lpr/lpr mice in CD4 TN cells, indicating the faster depletion of the subset in β-cat⁻flpr/lpr mice (Figures 4(b) and 4(c)). Another long live subset T cell, the Tₑₑₘₑₑ had no significant difference in four groups, either in CD4 or CD8 T cells (Figures 4(b) and 4(c)). These results showed that β-cat⁻fl accelerated the depletion of naïve CD4 T cell and affected the formation of effector memory T cell in different fashions.

3.4. Stabilized β-Catenin Declined the Levels of Serum Inflammatory Cytokines of lpr/lpr Mice. To analyze the production of cytokine and chemokine in serum, we used Bio-Plex Pro Mouse Cytokine 23-Plex panel was used in this study. It is obvious that the concentrations of IFN-γ, IL-12(p40), IL-17, IL-5, IL-10, and IL-13 were elevated in lpr/lpr mice compared with WT mice and rescued by β-cat⁻fl in β-cat⁻flpr/lpr mice (Figure 5(a)). In addition, we found that the amount of IL-2 was obviously increased in β-cat⁻flpr/lpr mice compared with lpr/lpr mice (Figure 5(a)). Meanwhile, the inflammatory factors were detected, including IL-1α, IL-1β, IL-6, TNF-α, and GM-CSF, all displayed a lower level in β-cat⁻flpr/lpr mice than lpr/lpr mice, but they were obviously elevated in lpr/lpr mice compared with WT mice (Figure 5(b)). The inflammatory-related chemokines KC, MCP-1, MIP-1α, MIP-1β, RANTE, and Eotaxin also were detected; it can be seen that the levels of them, except for KC, were significantly elevated in lpr/lpr mice compared with WT mice (Figure 5(c)). Whereas, they were obviously declined in β-cat⁻flpr/lpr mice compared with lpr/lpr mice (Figure 5(c)). These results demonstrated that β-cat⁻fl could reduce the levels of serum inflammation-related cytokines and chemokines of lpr/lpr mice.

3.5. Stabilized β-Catenin Slightly Ameliorated the Lupus Nephritis of lpr/lpr Mice. As is well-known, lupus nephritis has often been reported in lpr/lpr mice. The production and accumulation of autoantibodies was one of the important clinical biomarkers of lpr/lpr mice [23]. Thus, the levels of serum pathogenic anti-dsDNA antibodies were detected in all mice by using the enzyme-linked immunosorbent assay (ELISA). Consistent with previous reports, the level of anti-dsDNA autoantibody was significantly increased in lpr/lpr mice compared with WT mice (Figure 6(b)). Nevertheless, β-cat⁻fl slightly declined the level of anti-dsDNA autoantibody of lpr/lpr mice (Figure 6(b)). In the present study, the kidneys of WT, β-cat⁻fl, lpr/lpr, and β-cat⁻flpr/lpr mice were processed and stained with H&E and PAS for histomorphologic changes detecting (Figure 6(a)). It is obvious that β-cat⁻flpr/lpr, lpr/lpr, and β-cat⁻fl mice all showed apparently severe kidney damages which were characterized with the mesangial matrix expansion of glomerular, the mesangial matrix increases of glomerulus, the inflammatory infiltration of glomerulus, the capillary basement membrane thickening of glomerulus, the crescent formation, the inflammatory infiltration of interstitial, the edema of interstitial, the local sclerosis of vascular, and the inflammatory invasion of perivascular compared with WT mice (Figure 6(a)). The histopathologic scores of β-cat⁻flpr/lpr, lpr/lpr, and β-cat⁻fl mice were significantly higher than WT mice, but β-cat⁻flpr/lpr mice had a relatively lower histopathologic score than lpr/lpr mice (Figure 6(c)). In addition, we performed a fine grade histopathologic score evaluation which include eight subclinical characters and most of them showed the same tendency that β-cat⁻fl slightly declined the scores of β-cat⁻flpr/lpr mice (Figure 6(d)). These data implicated that β-cat⁻fl slightly ameliorate the lupus nephritis of lpr/lpr mice.

4. Discussion

It is well known that postthymic mature T cells express the T cell receptor α and β chains (TCRα/β) and also express either CD4 or CD8 surface glycoproteins. Some mature T cells express neither CD4 nor CD8, which were called “double-negative” cells and normally express another form of T cell receptor consisting of γ and δ chains [24]. However, some studies have identified a very rare subpopulation of T cells which express the TCRα/β but do not express CD4 or CD8 (TCRα/β CD4⁺ CD8⁺) in lpr/lpr mice and can induce autoimmune lymphoproliferative syndrome (ALPS) [13]. The accumulation of CD3⁺ T cell receptor (TCR)αβ⁺ CD4⁺ CD8⁻ double-negative T cells (DNT) is a hallmark of autoimmune lymphoproliferative syndrome (ALPS) [25]. Many genetic mutations and the regulator overexpression including Fas, Fasl, LPS-responsive and beige-like anchor (LRBA), miR-146a, and IL-12RB1 could result in an ALPS-like phenotype [26–28]. As an incurable disease, ALPS treatments mainly focus on the obliteration of autoimmune disease, lymphadenopathy, lymphoma, and other concurrent diseases. Except for high-dose corticosteroids as common therapy, mammalian target of rapamycin (mTOR) inhibitors sirolimus and pentostatin, Rituximab, and mycophenolate mofetil (MMF) have also been successfully applied in some patients [10]. Many documents have identified ALPS as an example of a genetic disorder of lymphocyte apoptosis caused by mutations in the genes which are related to Fas-mediated cell death pathway, but only few therapeutical strategies for ALPS are relatively known. Indeed, except for the Fas-mediated apoptotic pathway deficiency in ALPS, the role of the
Figure 4: Stabilized β-catenin affected the formation of memory T cells. All female animals were sacrificed at age 6 months, and splenocytes were isolated and stained with indicated surface markers. (a) The representative FACS results of memory T cells subpopulation, gating on CD4 and CD8 positive T cell. (b) The numbers of TE, TEM, TCM, and TN cells in CD4 T cells are shown. (c) The numbers of TE, TEM, TCM, and TN cells in CD8 T cells are shown. Data are shown as the mean ± SD (n = 6–10). *p < 0.05; **p < 0.01; and ***p < 0.001.
intrinsic apoptotic pathway or others in ALPS pathophysiology is not known. β-Catenin is a multifunctional protein and is the most important information molecule in the canonical Wnt pathway to regulate cell growth, differentiation and apoptosis, and so forth [6]. Previous studies have shown that β-catenin regulates the function of CD8 T cell, and the generation and function of CD8 memory-like T cell also are induced by β-catenin transgene [7]. The canonical Wnt/β-catenin pathway had been reported to regulate multiple functions, including stem cell regeneration and kidney and reproductive system organogenesis processes [29]. In the present study, we demonstrated that stabilized β-catenin slightly rescued some Fas-mutation-mediated ALPS-like manifestations, especially the double-negative T (TCRβ⁺CD4⁻CD8⁻) cells, by regulating the Fas-independent-mediated apoptosis pathway, uncovering a potential novel therapeutic direction for ALPS.

Previously, we have shown that, in response to staphylococcal enterotoxin B stimulation, Fas crosslinking, or cytokine withdrawal, the T cells from β-catenTg mice were more sensitive to apoptosis compared with WT mice by binding to Fas promoter to stimulate it and that the levels of Fas on activated T cells were significantly higher in β-catenTg mice than in WT mice, suggesting that stabilized β-catenin was able to promote the apoptosis of activated T cells in part by upregulating the expression of Fas on activated T cells [20]. In the present study, we introduced stabilized β-catenin into lpr/lpr mice, to examine the effects of the stabilized β-catenin on the survival of peripheral mature T cells in the state of Fas deficiency. We carried out the activation-induced cell death

**Figure 5:** Stabilized β-catenin decreased serum inflammatory cytokine level of lpr/lpr mice. All female animals were sacrificed at age 6 months, and serum were collected, then multiple cytokines level were detected by multiple enzyme-linked immunosorbent assay kit. (a) The levels of comparison of Th1, Th17, Th9, and Th2 cells-related cytokines in serum are shown. (b) The levels of comparison of proinflammatory cytokines and colony-stimulating factors in serum are shown. (c) The levels of comparison of chemokines in serum are shown. Data are shown as mean ± SD (n = 3–8). *p < 0.05; **p < 0.01.
Figure 6: Lupus nephritis was slightly relieved in β-cat\textsuperscript{Tg}lpr/lpr mice. All female animals were sacrificed at age 6 months. (a) H&E and PAS staining were used for renal pathology examine. (b) The relative levels comparison of anti-dsDNA antibody in serum. (c) Histopathologic score is shown according to H&E and PAS staining results. (d) The fine grade histopathologic scores for four different groups are shown. Data are shown as mean ± SD (n = 6–10). *p < 0.05; **p < 0.01; and ***p < 0.001.
(AICD) experiment by anti-CD3 Ab stimulation in vitro and found that β-cat^{fl} could not only potentiate Fas-mediated T cell apoptosis, but also potentiate the T cell apoptosis in lpr/lpr mice (Figure 2). We also demonstrated that β-cat^{fl} slightly ameliorated the ALPS-like symptoms of lpr/lpr mice most likely via Fas-independent signal-mediated apoptosis in vivo. Stabilized β-catenin partly reduced the numbers of DNT (TCRβ^{+}CD4^{+}CD8^{−}) and T_{EM} cells in lpr/lpr mice (Figures 3 and 4). Especially, β-cat^{fl} obviously reduced the levels of serum inflammation-related cytokines and chemokines in lpr/lpr mice (Figure 5). Thus, β-catenin pathway regulates the survival of peripheral mature T cells by promoting apoptosis in two different mechanisms at least.

Regulated induction of apoptosis is required to maintain T cell homeostasis which is critical for normal functioning of the immune system. In C57BL/6 background lpr/lpr mice, we found that they showed the ALPS and lupus-like disease symptoms, including progressive accumulation of TCRβ^{+}CD4^{+}CD8^{−} T (DNT) cells in spleen, autoantibody production, peripheral mature CD4^{+} and CD8^{+} T cells proliferation, lupus nephritis, and other autoimmune phenomena consistent with previous studies [9, 17, 30]. As a hallmark of the disease, DNT cells can induce autoantibody production, proinflammatory cytokines secretion, and inflamed tissue infiltration [31]. The present of high level autoantibodies to anti-dsDNA also have been linked to the disease progression of SLE [32]. We also found that lpr/lpr mice had aberrant transcriptional programs and differentiation of CD4^{+} and CD8^{+} T cell subsets, and Fas-negative T cells accumulated not only among DNT, but also among CD4^{+} and CD8^{+} T_{EM} cells, which is consistent with previous studies in ALPS patients [25]. DNT-like cells were detected in both CD4^{+} and CD8^{+} T_{EM} populations and the accumulation of these cells before their double-negative state appears to be an important early event in the pathogenesis of lymphoproliferation in ALPS patients, indicating that DNT can not only derive from CD8^{−}, but also from CD4^{+} T cells [25]. Our study also revealed that the numbers of T_{S} cells, such as CD4 or CD8, in β-cat^{fl}lpr/lpr, lpr/lpr, and β-cat^{fl} mice were dramatically decreased compared with WT mice. However, in β-cat^{fl}lpr/lpr mice, the CD4 T_{S} cells displayed faster depletion and the numbers of DNT (TCRβ^{+}CD4^{+}CD8^{−}) and T_{EM} cells all had a obvious decline compared with lpr/lpr mice. These results indicated that β-cat^{fl} partly ameliorated the ALPS-like manifestations of lpr/lpr mice.

In lpr/lpr mice, SLE also is a manifestation of ALPS. We all know that cytokines play an important role in the pathogenesis of SLE and their balance determines disease activity. For example, the serum IL-6 and IL-10 levels correlate with disease activity [33, 34]. The balance between Th1 cytokines and Th2 cytokines in SLE has been shown to be involved in the pathogenesis of inflammation [35]. In the present study, we found that lpr/lpr mice had higher IFN-γ, IL-12(P40), IL-10, and IL-6 levels in serum than WT mice, which is consistent with previous studies in SLE patients [33]. The levels of IL-17 and IL-13 also had an obvious elevation in lpr/lpr mice serum compared with WT mice. We also found that proinflammatory cytokine IL-1β and TNFα were boosted during the process of disease in lpr/lpr mice serum consisting with previous studies [36]. In addition, the chemokine MIP-1α (CXCL3), MIP-1β (CXCL4), RANTES (CXCL5), and Eotaxin (CXCL11) also had higher levels. Chemokines are a group of cytokines, whose main action is to recruit leukocyte subsets under homeostatic and pathological conditions. These chemokines play important roles during the inflammation; for example, MIP-17α (CXCL3) and RANTES (CXCL5) can bind to receptor CXCR5 and can circulate and migrate immune cells such as neutrophils, monocytes, and effector T cells into inflamed tissues [37]. Over the past decade, the concept of targeting cytokines to treat autoimmune diseases has evolved at a rapid pace, such as some medicines approved by the drug administration to target IL-1β, IL-5, IL-6, IL-2, TNF-α, and IFN-γ [38]. In fact, we found that the levels of IFN-γ, IL-12(P40), IL-10, IL-6, IL-17, IL-13, IL-1β, TNFα, MIP-1α, MIP-1β, RANTES, Eotaxin, and GM-CSF all have a decline in β-cat^{fl}lpr/lpr mice serum compared with lpr/lpr mice. Previous study showed that Th1, Th2, and Th17 cells according to the unique functions, respectively, produced IFN-γ, IL-4, and IL-17 cytokines [39, 40]. Meanwhile, Th1 and Th17 cells contribute to kidney injury in renal inflammatory diseases [41]. These data suggested that β-cat^{fl} ameliorated the inflammation of lpr/lpr mice by downregulating multiple inflammatory cytokines/chemokines, and this could be the result of Th1, Th2, and Th17 effects or a combination of effects.

In the present study, the C57BL/6 background lpr/lpr mice also showed higher concentration of the autoantibody and lupus nephritis, which were the manifestations of SLE [42]. However, β-cat^{fl}lpr/lpr mice demonstrated slightly different compared with lpr/lpr mice. In addition, we found that β-cat^{fl} mice demonstrated more severe lupus nephritis, but with a lower concentration of the autoantibody than WT mice. While, previous studies have reported that Wnt/β-catenin has a critical role in cell motility and extracellular matrix (ECM) accumulation in tissue fibrosis, and sustained activation of Wnt/β-catenin signaling in adult kidney is associated with glomerular and tubulointerstitial epithelial-mesenchymal transition (EMT), leading to the development and progression of renal fibrotic lesions in chronic kidney diseases [43, 44]. At this point, additional studies are required to further identify the exact temporal and spatial effects of stabilized β-catenin in renal injury. In summary, β-cat^{fl} and lpr/lpr mice all showed severe lupus nephritis, but β-cat^{fl}lpr/lpr mice demonstrated relatively slight lupus nephritis (Figure 6). These results demonstrated that β-cat^{fl} ameliorated lupus nephritis of lpr/lpr mice.

5. Conclusions

In summary, we reported herein that stabilized β-catenin partly relieved the augments of splenic lymphocytes, the inflammation, and the lupus nephritis of Fas-mutation-mediated ALPS-like manifestations of lpr/lpr mice, especially suppressed the DNT (TCRβ^{+}CD4^{+}CD8^{−}) and T_{EM} cells, and declined the levels of some serum inflammation cytokines and chemokines most likely via Fas-independent signal pathway-mediated apoptosis. These findings suggested that stabilized β-catenin, in some extent, ameliorated ALPS-like...
symptoms of lpr/lpr mice and might uncover a potential novel therapeutic direction for ALPS.

**Abbreviations**

- β-catTg: Stabilized β-catenin by genetically modified GFP expression
- ALPS: Autoimmune lymphoproliferative syndrome
- AICD: Activation-induced cell death
- lpr: Lymphoproliferation
- DNTs: Double-negative T cells
- SLE: Systemic lupus erythematosus
- ELISA: Enzyme-linked immunosorbent assay
- T_{E}: Effective T cell
- T_{EM}: Effective memory T cell
- T_{CM}: Center memory T cell
- T_{N}: Naïve T cell
- H&E: Hematoxylin
- PAS: Periodic acid Schiff
- TNFα: Tumor necrosis factor α
- IFN-γ: Interferon gamma

**Ethical Approval**

All procedures and experiments involving animals in this study were reviewed and approved by the Ethics Committee of the Zhongshan School of Medicine, Sun Yat-sen University (Guangzhou, China).

**Conflicts of Interest**

The authors declare that they have no competing interest.

**Authors’ Contributions**

Xiaoxie Xu and Zhaofeng Huang designed the study. Xiaoxie Xu, Jun Huang, and Mei Zhao performed the experiments and collected the data. Bolan Yu and Qiao Su provided the reagents. Xiaoxie Xu, Huanpeng Chen, Jinhua Mo, Xiaoqing Zhou, and Bolan Yu analyzed all the results. Xiaoxie Xu drafted the manuscript. All authors revised and approved the manuscript.

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