International Immunopharmacology 80 (2020) 106239

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Toll-like receptor 7 agonist imiquimod prevents the progression of SLE in MRL/lpr mice via inhibiting the differentiation of T follicular helper cells

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A R T I C L E I N F O

Keywords:
Toll-like receptor 7
T follicular helper (Tfh) cells
SLE
MRL/lpr mice
Immune tolerance

A B S T R A C T

Previous research has recently indicated that TLR7 is able to induce CD4$^+$T cell anergy, which is the opposite of the role it plays in innate immune cells. Therefore, TLR7 ligands may be used as a manner in which to induce CD4$^+$T cells "tolerance" in autoimmune diseases. T follicular helper (Tfh) cells were demonstrated to be a subset of CD4$^+$T cells that help B cells produce antibodies. The abnormal activity of Tfh cells, though, is their function as a primary pathogenic factor in systemic lupus erythematosus (SLE). However, the role of TLR7 in Tfh cells is not clear. Our study was aimed at determining the influence of TLR7 on Tfh cells in a murine model of SLE (MRL/lpr mice). We were surprised to find that the frequency of Tfh cells and germinal center (GC) B cells was significantly reduced after treatment with the TLR7 agonist imiquimod. Imiquimod also significantly reduced the expression of inducible costimulatory molecule (ICOS) and programmed death 1(PD-1) in Tfh cells and decreased IL-21 secretion. Moreover, imiquimod significantly reduced the mRNA expression of several transcription factors, including *Bcl-6*, c-*Maf*, *Batf3*, *Nfatc2* and *Stat3,* and enhanced the expression of *Prdm1* and *Stat5b* in CD4$^+$T cells. Imiquimod also ameliorated the progression of SLE in MRL/lpr mice by inhibiting anti-dsDNA antibodies and antinuclear antibody (ANA) secretion in the serum. Our findings indicated that TLR7 inhibited the development of Tfh cells both *in vivo* and *ex vivo*, which depended on many transcription factors aside from *Bcl-6*. Our results demonstrated that a TLR7 agonist has the potential to be used to inhibit Tfh cell responses during SLE.

1. Introduction

Toll-like receptors (TLRs) act as a bridge between microorganisms and host cells by recognizing specific pathogen-associated molecular patterns (PAMPs) expressed in distinct microorganisms [1–5]. Interestingly, some TLRs have been found to be expressed on T cells [6]. PAMPs can induce the survival of activated CD4$^+$T cells, which suggests that TLRs on T cells can directly modify adaptive immune responses [7–11]. However, a recent report has elucidated that the function of TLR7 in CD4$^+$T cells is in absolute opposition to the role it plays in innate immune cells, which has described a new pathway mediated by TLR7 that suppresses the activity of CD4$^+$T cells [12]. By inducing a calcium flux in CD4$^+$T cells, TLR7 can induce anergy in these cells [12]. The activation of the genes involved in inducing anergy was dependent on the transcription factor *Nfatc2* and resulted in subsequent T cell unresponsiveness [12].

Although the function of TLR7 in T cell activation has been extensively studied, little is known about the stimulatory effect of TLR7 on Tfh cells. Located in the germinal centers (GC), Tfh cells play important roles in the regulation of the adaptive immune response [13]. There are some surface molecules that are highly expressed on the surface of Tfh cells, including C-X-C chemokine receptor type 5 (CXCR5), programmed death-1 (PD-1), and inducible costimulatory (ICOS) molecule [14–18]. The function of Tfh cells is to regulate the differentiation of GC B cells to memory B cells and plasma cells. Unlike other CD4$^+$T cells, Tfh cells can express BCL-6 and produce functional

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https://doi.org/10.1016/j.intimp.2020.106239
Received 13 November 2019; Received in revised form 4 January 2020; Accepted 20 January 2020
1567-5769/ © 2020 Published by Elsevier B.V.

cytokines, including IL-21 [19–21]. $CD4^+CXCR5^+ICOS^{high}PD\text{-}1^{high}$ cells are defined as circulating Tfh cells and were found to be increased in the peripheral blood of newly systemic lupus erythematosus (SLE) sufferers. Tfh cells have long been implicated in SLE pathogenesis. In sanroque mice, the proportion of circulating Tfh cells has a strong correlation with their GC counterparts, which makes Tfh cells a feasible human biomarker for this novel mechanism of a breakdown in GC tolerance [22]. Both the regulation of Tfh cell differentiation and GC reactions may be potential regulatory mechanisms to sustain immune tolerance and prevent the progression of SLE [22].

In this study, we aimed to identify the influence of TLR7 on Tfh cells during SLE. To accomplish this, we utilized a murine model of SLE (MRL/lpr mice), which spontaneously develop a characteristic symptom similar to SLE in humans. We demonstrated that TLR7 mediated the negative regulation of the Tfh cell response. Our studies found that engaging TLR7 decreased the frequency of Tfh cells in MRL/lpr mice via regulating certain transcription factors, such as by *Bcl-6*, *Blimp-1*, and *Stats*. Our results elucidated the mechanism by which TLR7 inhibits the immune response, which occurs through the regulation of transcription factors, interference with GC B cells, and production of antibodies in the presence of signaling via TLR7.

2. Materials and methods

2.1. Mice

6–8 weeks old female MRL/lpr mice were purchased from the Shanghai SLAC Laboratory Aniaml Co., Ltd, and 6–8 weeks female old BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All mice were housed under specific pathogen- free conditions in the Ningxia Medical University Laboratory Animal centre of Ningxia Medical University. All mouse procedures were approved by the Institutional Animal Care and Use Committee of the Ningxia Medical University.

2.2. $CD4^+$T cell purification and Tfh cell differentiation

Splenocytes were obtained from BALB/c mice (6–8 weeks old; female). Total $CD4^+$T cells were isolated by negative selection using a $CD4^+$T cell isolation kit (19852; StemCell Technologies). Purified $CD4^+$T cells were activated in 96-well plates (NY14831; Costar) coated with a CD3ε monoclonal antibody (mAb) (1.0 μg/mL) for 16 h. For the differentiation of Tfh cells, IL-21 (594-ML-010/CF;R&D SYSTEMS) (10 ng/mL), IL-6 (554582; BD Biosciences) (10 ng/mL), and CD28 mAb (553294; BD Biosciences) (1.0 μg/mL) were added to the cells with or without imiquimod at concentrations of 0.1, 1, 2.5, or 5 μg/mL (99011-78-6; Invivogen). At day 3, the cells were collected for flow cytometry and Taqman PCR (q-PCR), and the supernatants were collected for analysis via ELISA.

2.3. Murine experimental model

To examine the response of Tfh cells to a TLR7 agonist *in vivo*, MRL/lpr and BALB/c mice (16 weeks old; female) were randomized into two groups. Each group received intraperitoneal injections every other day for 4 weeks as follows: (1) 0.05 mg/kg of imiquimod (Purity $>$ 99%, 99011–02-6; MedChem Express), a compound of the imidazoquinadine family and a TLR7 agonist, in 100 μL of normal saline of 1% DMSO (D2650;Sigma-Aldrich, Steinheim, Germany); (2) 100 μL normal saline of 1% DMSO. All of the mice were euthanized at the age of 20 weeks. Blood, spleen, and kidney tissues were collected for future analysis.

2.4. Flow cytometry analysis

For the detection of the proportion of Tfh cells and costimulatory molecules of Tfh cells, total $CD4^+$ T cells were isolated by negative selection using a $CD4^+$ T cell isolation kit. The isolated cells were then stained with a FITC-conjugated anti-CD4 antibody (553047; BD Biosciences), APC-conjugated anti-CXCR5 antibody (560615; BD Biosciences), PE-conjugated anti-PD-1 antibody (551892; BD Biosciences, and BV421-conjugated anti-ICOS antibody (564070; BD Biosciences) for 30 min, followed by PerCP-Cy5.5- conjugated anti-BCL-6 intracellular staining (563581; BD Biosciences) using a FoxP3 staining kit (00-5523-00; eBioscience). For the detection of GC B cells in mice, PBMCs were stained with an AF488-conjugated anti-B220 antibody (557669; BD Biosciences) and AF647-conjugated anti-GL-7 antibody (561529; BD Biosciences) for 30 min. For the detection of IgG1 plasma cells in mice, PBMCs were stained with a FITC-conjugated anti-CD19 antibody (557398; BD Biosciences) and PE-conjugated anti-IgG1 antibody (550083; BD Biosciences) for 30 min. Cell acquisition was performed on a FACSCelesta™ flow cytometer (BD FACSCelesta™).

2.5. Cytokine and autoantibody titer analysis using ELISA

Cytokines and antibody in supernatants or serum were measured by ELISA (IL-21 kit from (JL20239; J&l Biological) and others from eBioscience.

2.6. Taqman PCR analysis

RNA was isolated from $CD4^+$T cells using an RNeasy Micro Plus Kit according to the manufacturer's guidelines (DP419; Tiangen). RNA was converted to cDNA by reverse transcription with random hexamers and Multiscribe RT (#1622; TQMN, Reverse Transcription Reagents; Applied ThermoFisher). For mRNA expression assays, the following probes were used (all from Applied ThermoFisher):*Il-21*, Mm00517640_m1; *Bcl-6*, Mm00477633_m1; *c-Maf*, Mm02581355_s1; *Stat3*, Mm01219775_m1; *Batf3*, Mm01318274_m1; *Prdm1*, Mm00476128_m1; *Stat5a*, Mm03053818_m1; *Stat5b*, Mm00839889_s1; *Nfatc2*, Mm01240677_m1; *b-actin*, Mm02619580_m1. The reactions were set up following manufacturer's guidelines and were run on a StepOne Real-Time PCR System (Applied Biosystems). Values are presented as the difference in cyclineg threshold (Ct) values normalized to those of mRNA encoding β2-microglobulin for each sample, calculated by the following formula: relative RNA expression = $(2^{-\Delta Ct}) \times 10^3$.

2.7. Histopathology test

The kidneys were fixed with a 4% neutral-buffered formalin fixative (Top0382; Biotopped) overnight at 4 °C and then rinsed for 2 h in distilled H_2O. The kidneys were stored in 70% ethanol and then embedded in paraffin (LeicaB; 39601095). For histopathological examination, 4-μm-thick paraffin sections were stained with hematoxylin and eosin (HE).

2.8. Immunofluorescence evaluation of IgG deposits in kidney

Kidneys were embedded in OCT compound and snap-frozen at −70 °C. Frozen kidney sections (6 μm) were fixed in acetone, washed with phosphate buffered saline (PBS), blocked with 10% goat serum in PBS for 30 min, and stained with FITC-conjugated goat anti-mouse IgG (ab7064; R&D).

2.9. Statistics

All data were subjected to statistical analysis using Prism software version 6 (GraphPad). Comparisons between two groups were performed by 2-tailed *t*-test (parametric) or paired *t*-test (non-parametric). Comparisons between multiple groups were performed using a 1-way ANOVA with Bonferroni's multiple comparison tests. A $P < 0.05$ was considered signifificant.

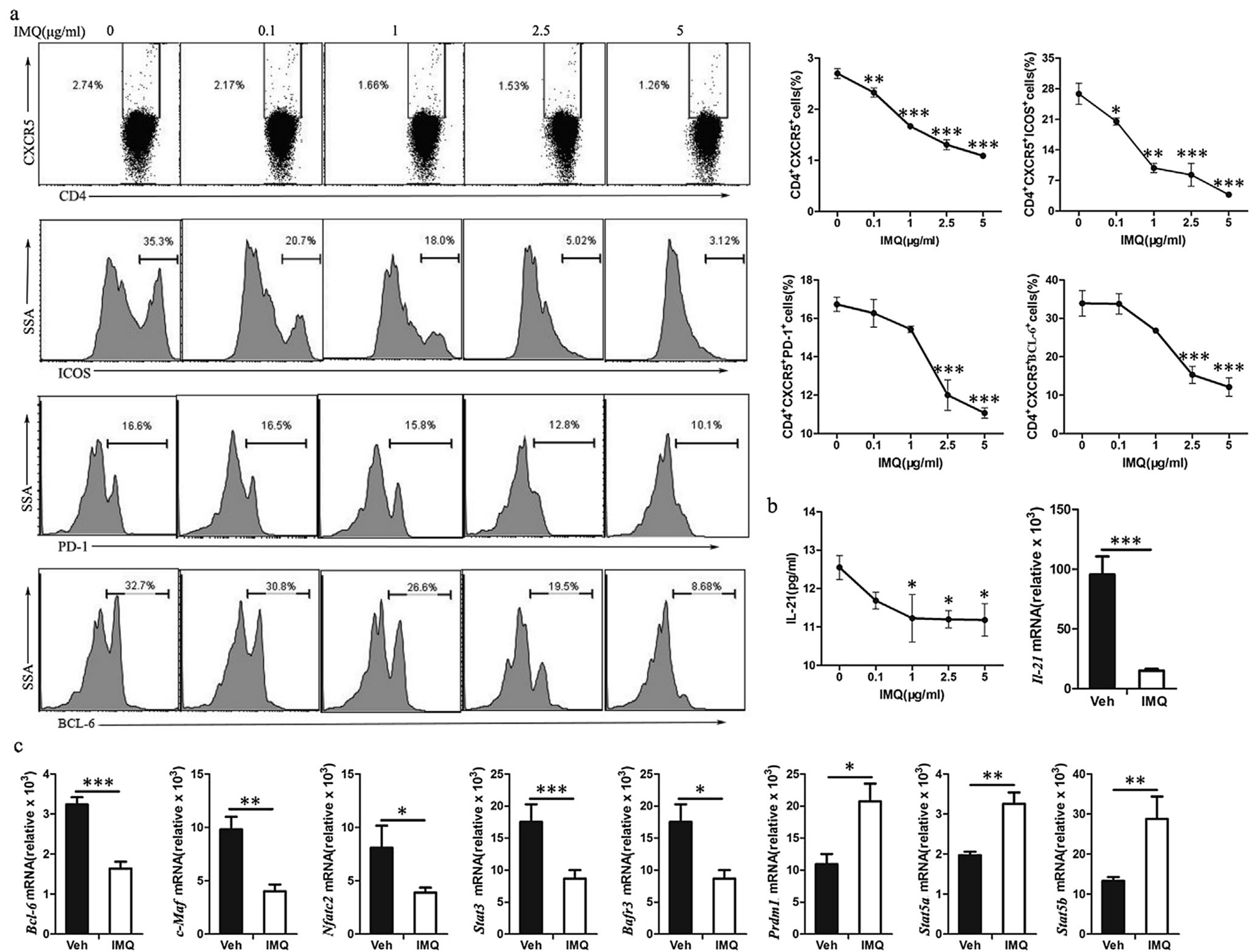

Fig. 1. Effects of TLR7 agonist imiquimod (IMQ) on Tfh cells *in vitro*. (a) The frequency of CD4$^+$CXCR5$^+$ lymphocytes, representative expression of ICOS, PD-1 and BCL-6 in Tfh cells after treatment with imiquimod *in vitro* by flow cytometry. (b) Cytokine expression in culture supernatants analyzed by ELISA, and mRNA expression of *Il-21* measured by Taqman-PCR. (c) mRNA expression of *Bcl-6, Batf3, c-Maf, Nfatc2, Stat3, Prdm1* and *Stat5* were measured by Taqman-PCR. Values are the mean and SD of 3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001.

3. Results

3.1. Imiquimod treatment reduced the response of Tfh cells in vitro

Purified CD4$^+$T cells were stimulated for 3 days with CD3εmAb, CD28 mAb, IL-21, and IL-6 in the presence or absence of the synthetic TLR7 agonist imiquimod (Supplementary Fig. 1). We analyzed the expression of molecules on Tfh cells by flow cytometry. Our results implied that the frequency of CD4$^+$CXCR5$^+$T cells was significantly decreased in a dose-dependent manner after intervention with imiquimod (Fig. 1a and Supplementary Fig. 2b). The expression of Tfh cell surface markers, such as ICOS and PD-1, was inhibited by imiquimod in a dose-dependent manner (Fig. 1a and Supplementary Fig. 2c-d). Tfh cell differentiation is critically dependent on BCL-6 expression; thus, we also analyzed the proportion of BCL-6 after treatment with the synthetic TLR7 agonist imiquimod (Supplementary Fig. 2e). Our results showed that treatment with imiquimod resulted in a significantly decreased expression of BCL-6 in Tfh cells as compared to that of vehicle-treated control cells (Fig. 1a). Additionally, the concentration of IL-21 was significantly decreased in a dose-dependent manner as compared to that of the vehicle control (Fig. 1b). We also analyzed the mRNA expression of *Bcl-6* and *Il-21*, which was consistent with the protein expression (Fig. 1b–c).

We also determined whether stimulation with imiquimod influenced other transcription factors associated with Tfh development and function. We analyzed the gene expression of several other transcription factors by qPCR after 72 h post-imiquimod treatment. It is important to note that imiquimod differentially downregulated *c-Maf*, *Batf3*, *Nfatc2*, and *Stat3* in CD4$^+$T cells. However, imiquimod stimulation upregulated the mRNA expression of *Prdm1* (which encodes *Blimp-1*, a transcription repressor that inhibits Tfh generation) and *Stat5a/5b* (Fig. 1c).

3.2. TLR7 signals minimized the response of Tfh cells in BALB/c mice

We treated BALB/c mice (female; 16 weeks of age) with imiquimod for 4 weeks and then examined if TLR7 signals influenced the response of Tfh cells *in vivo* (Supplementary Fig. 3). We found that the frequency of Tfh cells was significantly reduced after treatment with imiquimod (Fig. 2a and Supplementary Fig. 4). IL-21 has a critical effect on Tfh and B cells, including GC formation. Thus, we then measured the levels of IL-21 in the sera from BALB/c mice. Our results showed that the levels of IL-21 were downregulated after treatment with imiquimod (Fig. 2c). The expression of *Il-21* mRNA in CD4$^+$T cells was also decreased (Fig. 2c). We analyzed several transcription factors after treatment with imiquimod, and the results were consistent with our *ex vivo* results. The expression levels of *Bcl-6, c-Maf, Nfatc2,* and *Stat3* were downregulated; however, the expression levels of *Prdm1* and *Stat5a/5b* were

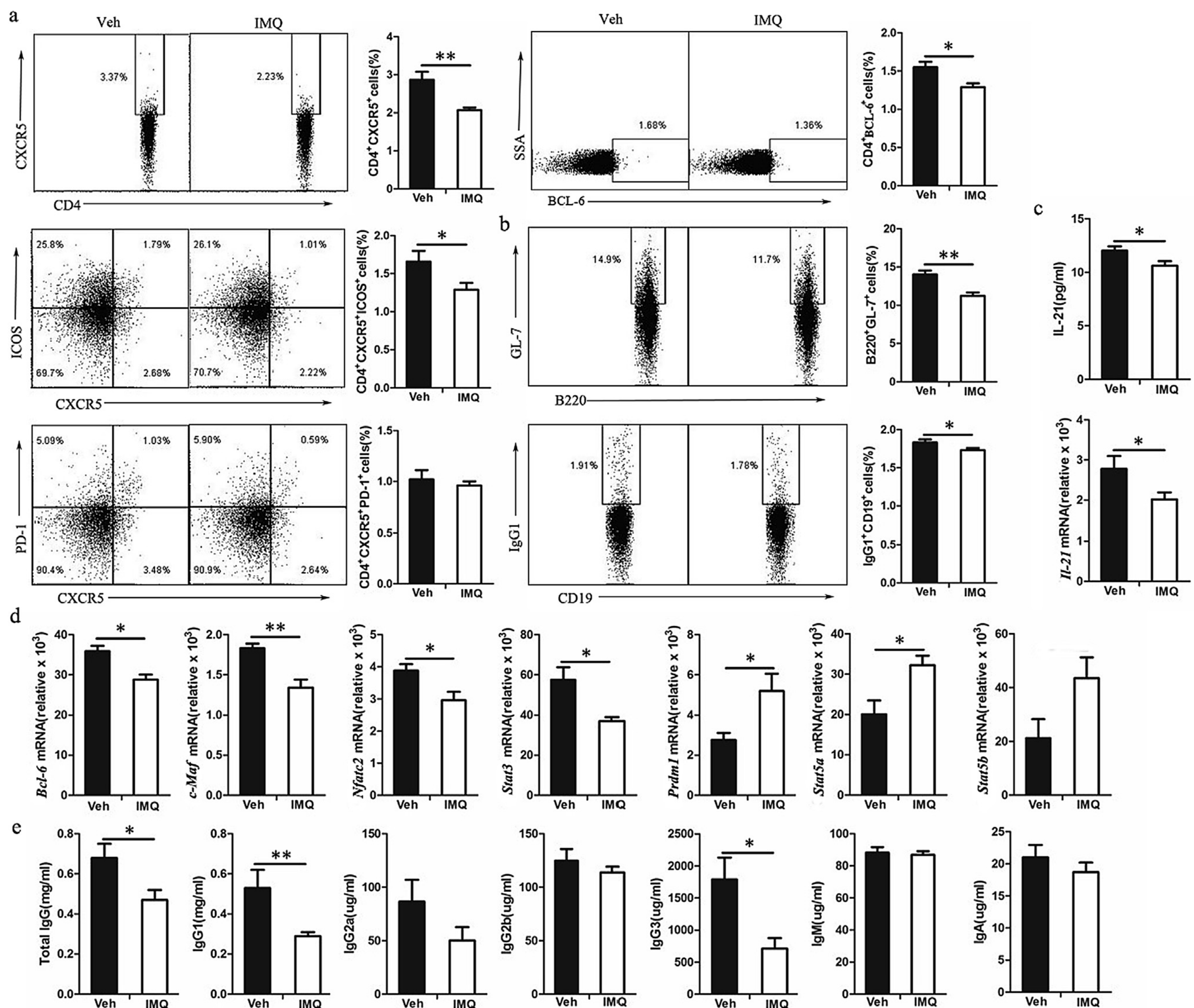

Fig. 2. Effects of TLR7 agonist imiquimod on Tfh cells, B cells response and antibody secretion in BALB/c mice. (a) The percentages of CD4$^+$CXCR5$^+$ lymphocytes, and representative expression of ICOS, PD-1 and BCL-6 Tfh cells in the spleen of BALB/c mice after treatment with imiquimod. (b) The percentages of B220$^+$GL-7$^+$GC B cells and gG1$^+$CD19$^+$B cells in splenocytes of BALB/c mice. (c) Cytokine expression in serum of BALB/c mice analyzed by ELISA, and mRNA expression of *Il-21* measured by Taqman-PCR. (d) mRNA expression of *Bcl-6, Batf3, c-Maf, Nfatc2, Stat3, Prdm1* and *Stat5* were measured by Taqman-PCR. (e) Levels of antibody in serum of BALB/c mice was analyzed by ELISA. Values are the mean and SD of 5 mice per group, *P < 0.05, **P < 0.01 and ***P < 0.001.

upregulated (Fig. 2d). *Batf3* was not detected. Taken together, these data suggested that TLR7 signals could downregulate the differentiation of Tfh cells.

Tfh cells have a direct effect on B cells and facilitate GC formation. Thus, we further examined the contribution of TLR7 signals to the development of B cell responses. Splenic GC formation was determined by the flow cytometry analysis of B220$^+$GL-7$^+$cells, which were described as GC B cells (Supplementary Fig. 5A). In parallel with effects on Tfh cells, we found the frequency of B220$^+$GL-7$^+$GC B cells and memory B cells in the spleen was significantly decreased after mice were treated with imiquimod (Fig. 2b). Consistent with the frequency of B cells, the levels of serum IgG, IgG1, and IgG3 were significantly reduced in mice treated with imiquimod. There was no effect on the levels of IgG2a/b, IgA, and IgM (Fig. 2e).

3.3. Imiquimod suppressed Tfh cells in MRL/lpr mice

In order to observe the potential biological and clinical relevance of our results, we detected whether stimulation of TLR7 with imiquimod could mediate Tfh cell differentiation in an SLE mouse model. Consistent with previous findings that autoimmune MRL/lpr mice exhibited splenomegaly with an expansion of Tfh cells in the spleen, our results revealed that MRL/lpr mice had a significantly higher proportion of CD4$^+$CXCR5$^+$cells as compared with BALB/c mice (Fig. 3a and Supplementary Fig. 4A-c). We also analyzed the expression of additional cellular molecules, including ICOS and PD-1. Our results showed significantly increased expression of ICOS and PD-1 in MRL/lpr mice as compared with BALB/c mice (Fig. 3a and Supplementary Fig. 4A-d/e). Expression of BCL-6 was also consistent with the frequency of Tfh cells (Fig. 3a and Supplementary Fig. 4B). IL-21 levels were increased in the sera of MRL/lpr mice as compared to BALB/c mice (Fig. 3b), and the mRNA expression of *Il-21* correlated with the protein results (Fig. 3b). This prompted us to examine whether the TLR7 agonist imiquimod could affect the expansion of Tfh cells. The results showed that the frequency of Tfh cells was significantly reduced in the spleen of mices treated with imiquimod (Fig. 3a). Furthermore, imiquimod also inhibited the expression level of IL-21 (Fig. 3b) in MRL/lpr mice.

Previous research has revealed a strong positive correlation between the increased number of Tfh cells and the pathogenesis and severity of disease in GC-dependent autoimmune conditions. MRL/lpr mice

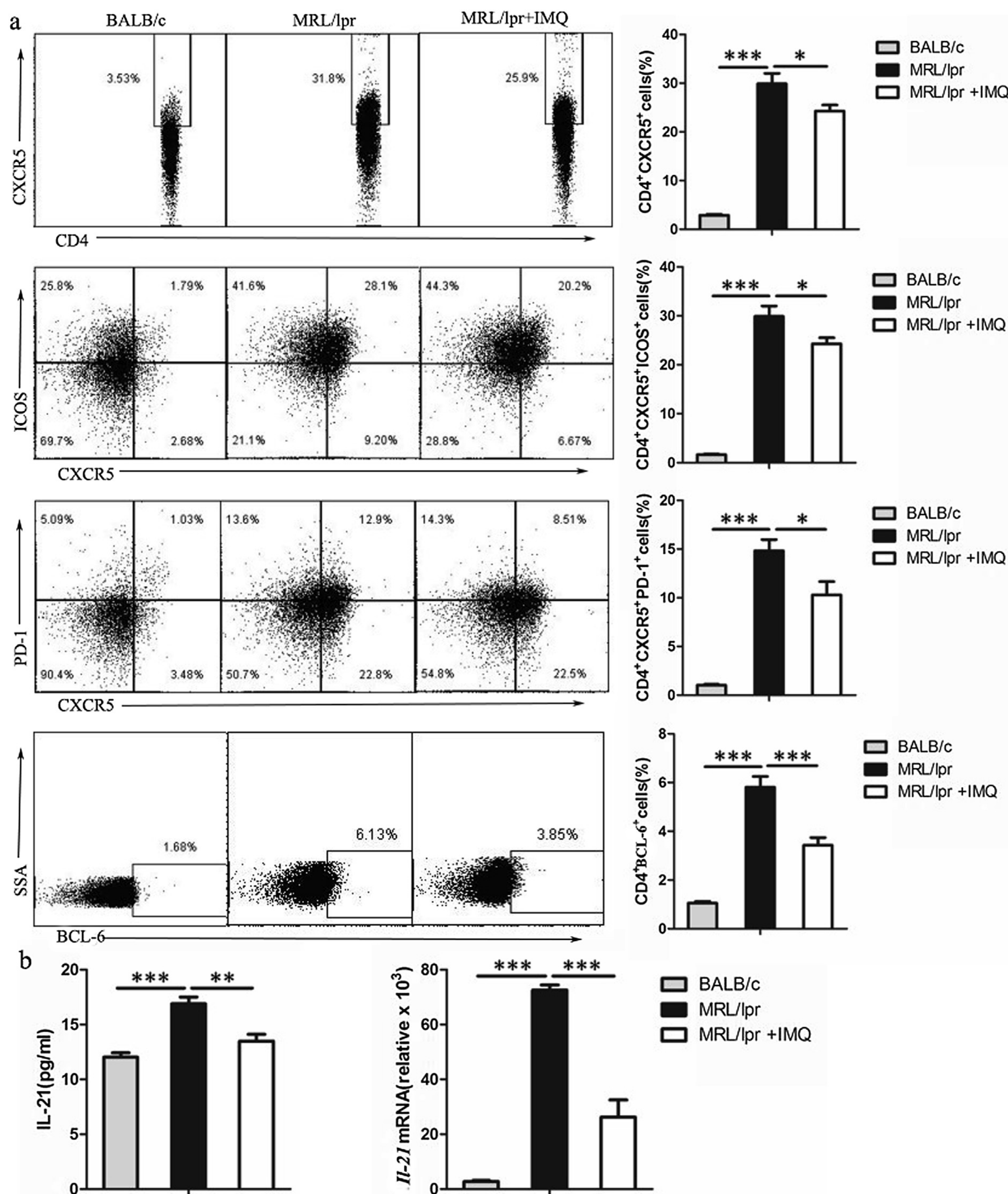

Fig. 3. TLR7 agonist imiquimod suppressed Tfh cells in MRL/lpr mice. (a) The percentages of CD4$^+$CXCR5$^+$ lymphocytes, representative expression of ICOS, PD-1 and BCL-6 Tfh cells in the spleen of MRL/lpr mice and BALB/c mice after treatment with imiquimod by flow cytometry. (b) Leves of Cytokine in serum of MRL/lpr mice and BALB/c mice was analyzed by ELISA, and mRNA expression of *Il-21* measured by Taqman-PCR. Values are the mean and SD of 5 mice per group. *P < 0.05, **P < 0.01 and ***P < 0.001.

exhibited splenomegaly (Fig. 4a), significantly increased IgG deposits in the glomeruli (Fig. 4c), significantly enhanced plasma levels of anti-dsDNA antibodies and ANA (Fig. 4b), and significantly enhanced levels of IgA, IgM, IgG, and IgG subtypes (Fig. 5b). We were surprised to find that severity of disease was reduced, and the levels of serum anti-dsDNA, ANA, IgG, IgG1, IgG2a, IgG3, IgA, and IgM (Figs. 4b and 5b) were significantly decreased in MRL/lpr mice after imiquimod treatment.

The development of GC B cells and antibody-producing plasma cells within the GC needs the help of Tfh cells. As such, we analyzed the levels of B cells and found that the number of Tfh cells in splenocytes was positively correlated with disease activity and B cells in MRL/lpr mice. Our results implied that the percentage of GC B cells (B220$^+$GL-7$^+$) and plasma B cells (CD19$^+$IgG1$^+$) was significantly higher in MRL/lpr mice as compared to BALB/c mice (Fig. 5a and Supplementary Fig. 5A/B), and these cell types were downregulated in MRL/lpr mice

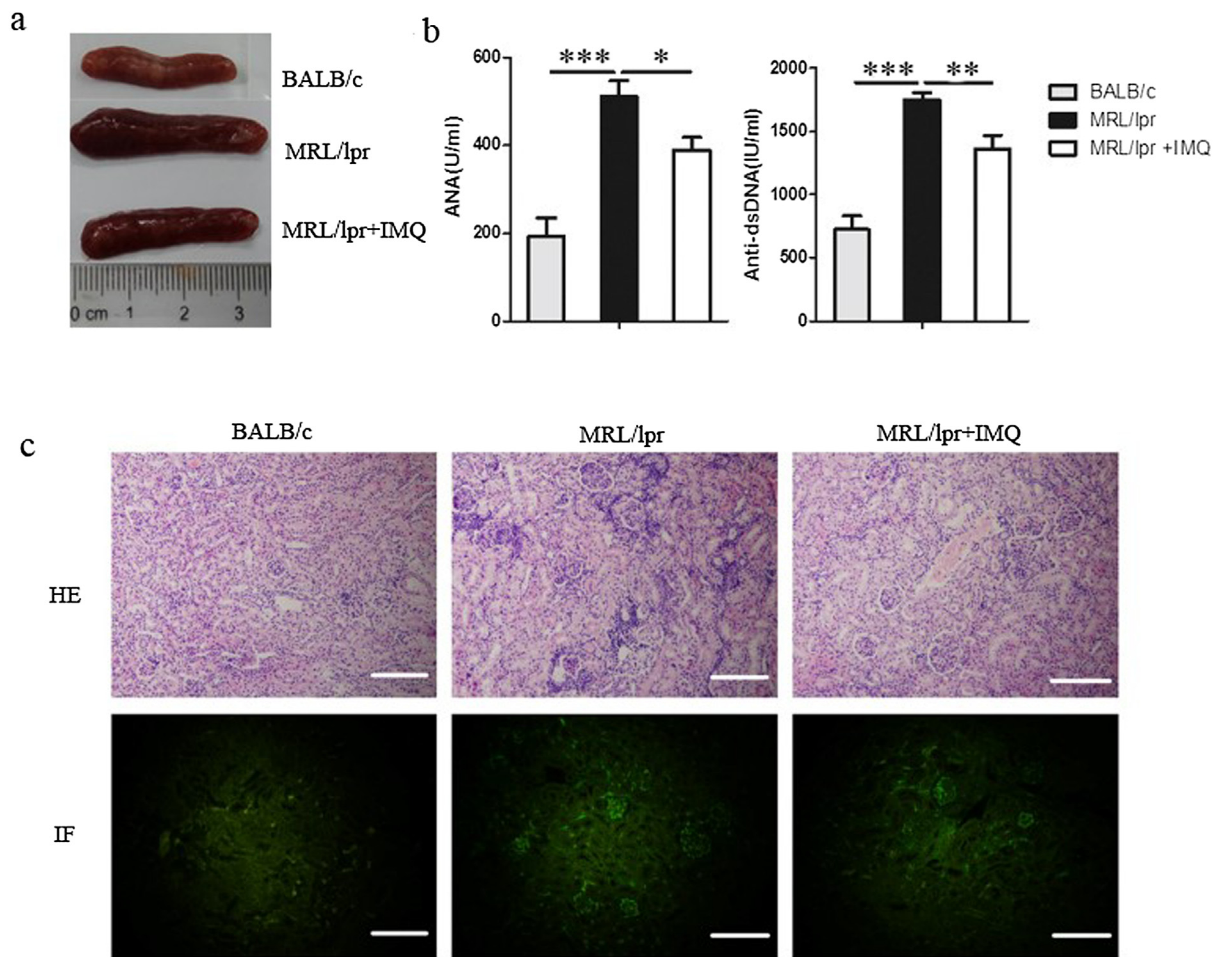

Fig. 4. TLR7 agonist imiquimod ameliorating the Progression of the SLE on MRL/lpr mice. (a) Splenomegaly in MRL/lpr mice and alleviated after treatment with imiquimod. (b) levels of anti-dsDNA antibodies, ANA, c-d, IgG deposits in glomeruli of MRL/lpr mice and BALB/c mice. Sections of kidney tissue were stained with H &E **c** and Immunofluorescence IgG. (d) Original magnification × 200. The scale bar in each image represents 100 μm. Values are the mean and SD of 5 mice per group, *P < 0.05, **P < 0.01 and ***P < 0.001.

after imiquimod treatment. We also analyzed the expression of several transcription factors and found that there was a significant down-regulation in the mRNA expression level of *Bcl-6, c-Maf*, *Batf3, Nfatc2,* and *Stat3* in the $CD4^+$ T cells of MRL/lpr mice, which was consistent with our results in Tfh cells after treatment with imiquimod. However, *Prdm1* and *Stat5b* were upregulated in MRL/lpr mice after treatment with imiquimod (Fig. 6).

4. Discussion

Recent studies have elucidated a previously undisclosed function for TLR7 in $CD4^+$T cells that is opposite to its role in innate immune cells [12]. Previous studies have revealed pathways involving the inhibition of the activation and function of $CD4^+$T cells by TLR7 [12]. As a newly identified $CD4^+$T cell subset, Tfh cells provide essential help to B cells, especially in the GC reaction [20,22–24]. Therefore, it is likely that Tfh cells exacerbate the pathogenesis of autoimmune diseases [25–27]. Moreover, TLR7 ligands may act as a target to inhibit Tfh cells during human autoimmune disease. Here, we demonstrated that activation of TLR7 in $CD4^+$T cells by its agonist imiquimod decreased the number of Tfh cells. As is known, Tfh cells assist B cells by expressing certain surface molecules, including ICOS, PD-1, and cytokine IL-21, which are crucial for GC B cells [28–31]. In this study, our results showed that the treatment of TLR7 in $CD4^+$T cells resulted in an obvious decrease in the levels of ICOS and PD-1 on Tfh cells. Moreover, our results also showed a decrease in IL-21 upon stimulation of TLR7. *Bcl-6* plays a predominant role in regulating Tfh cells and is repressed by the transcription factor of *Blimp-1*. The antagonizing interaction of *Bcl-6* with *Blimp-1* is essential for T cell differentiation [32–34].Our results showed that stimulation of TLR7 decreased expression of *Bcl-6*, but increased expression of *Prdm1,* which encodes *Blimp-1* [35]. Also, aside from *Bcl-6*, other transcription factors have also been indicated as the moderators of Tfh cell differentiation. Transcription factors that are positive moderators of Tfh cell differentiation include *Stat3, Batf, c-Maf*, and *Nfatc2* [36], and transcription factors that are negative moderators include *Stat5* and *Blimp-1* [37–42]. Our findings showed that all of the above transcription factors could be regulated by the stimulation of TLR7.

Our results have potential clinical implications, as some studies have implied that the aberrant differentiation of Tfh cells is significantly correlated with SLE pathogenesis. Additionally, aberrant Tfh cell activity leads to the pathogenesis of SLE through abnormal GC formation and massive anti-dsDNA and ANA production [19,43,44].

Tfh cells induce these phenomena via cytokines and co-stimulatory molecules, which stimulate B cells [19]. Thus, Tfh cells should be strongly associated with preventing autoimmunity by restricting GC reactions to self-antigen. Here, we found that MRL/lpr mice showed a reduction in renal injury and levels of autoantibodies after treatment with the TLR7 agonist imiquimod. We demonstrated that autoimmune phenotypes were alleviated when Tfh cell differentiation was down-regulated in MRL/lpr mice after treatment with the TLR7 agonist imiquimod. Although there are few studies regarding TLR signaling in Tfh cells, our study showed a previously undescribed negative effect of the

Fig. 5. TLR7 agonist imiquimod suppressed B cells in MRL/lpr mice. (a) The percentages of $B220^+GL\text{-}7^+$B cells and $IgG1^+CD19^+$B cells in splenocytes of MRL/lpr mice and BALB/c mice. (b) Levels of IgG, IgG subset, IgM and IgA in serum of MRL/lpr mice and BALB/c mice were analyzed by ELISA. Values are the mean and SD of 5 mice per group, *P < 0.05, **P < 0.01 and ***P < 0.001.

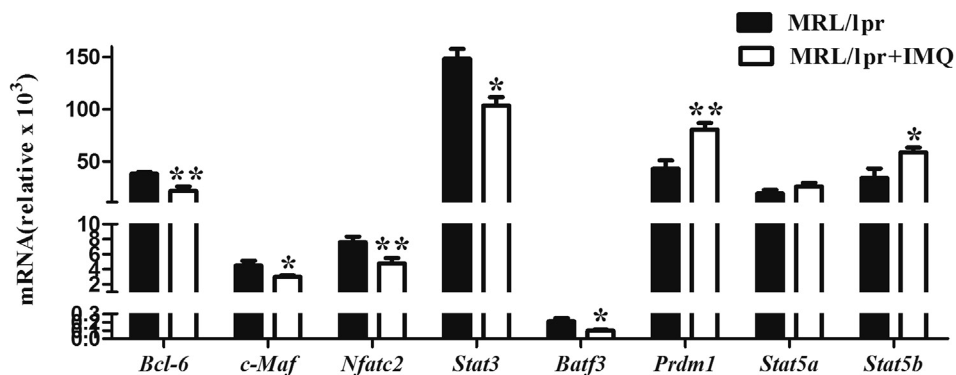

Fig. 6. mRNA relative expression of transcription factors in $CD4^+$T cells from spleen in MRL/lpr mice. mRNA expression of *Bcl-6, Batf3, c-Maf, Nfatc2, Stat3, Prdm1* and *Stat5* were measured by Taqman-PCR. Values are the mean and SD of 5 mice per group, *P < 0.05, **P < 0.01 and ***P < 0.001.

TLR signaling pathway on Tfh cell differentiation and IL-21 secretion, which was shown to be upregulated in SLE patients as compared to healthy controls. Furthermore, IL-21 expression was reported to elevate the production of Tfh-related transcription factors, such as *Batf*, in human SLE patients. Studies have elucidated that the development of Tfh cells is partly controlled by *Batf*-mediated direct regulation of *Bcl-6* and *c-Maf*, while the latter is essential for IL-21 production. Also, *Batf* has an effect on GC development by promoting the generation of Tfh, and GC B cell *Stat*-mediated cytokine signaling pathways are also important regulators of T helper cell development [38]. *Stat3* is thought to be crucial for Tfh cell differentiation [38]. *Stat5* efficiently suppresses Tfh differentiation by decreasing the mRNA expression of Tfh-associated genes, such as *c-Maf, Bcl-6, Batf,* and *Il-21* [38]. As a Tfh Inhibitor, *Blimp-1* can suppress the expression of *Bcl-6*, c-*Maf, Cxcr5*, and *Pd-1* [40]. *Blimp-1* can also decrease PD-1 expression by directly suppressing the role of *Nfat2* or by replacing *Nfat2* bound to the *Pdcd1* gene. Though there are numbers of *Nfat2* expressed in Tfh cells, little is known about the functions of *Nfat* family members in Tfh cells. NFAT proteins are important T cell receptors and Ca^{2+}-dependent regulators of T cell biology, supporting the major positive roles that NFAT family members play in Tfh differentiation [45–49]. Our results suggest that TLR7 inhibits Tfh cell differentiation, most likely by regulating these transcription factors.

In conclusion, we have disclosed a new role for TLR7 in Tfh cell function that is completely opposite to its role in innate immune cells. This may represent a novel mechanism by which PAMPs inhibit adaptive immune responses. Moreover, the TLR7 agonist imiquimod may be used to induce “tolerance” in Tfh cells during human autoimmune diseases.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81560504 grant to Dr Su, No. 81860138 grant to Dr Wu) ; Ningxia High School first-class Disciplines (West China first-class Disciplines Basic Medical Sciences at Ningxia Medical University, NXYLXK2017B07 grant to Dr Su).

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106239.

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