Glucocorticoid prevents CD138 expression in T cells of autoimmune MRL/*lpr* mice

TIANHONG XIE[1], HUIQIANG LIU[2] and PING LI[1]

[1]Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing Institute of Chinese Medicine; [2]Department of Pathology, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing 100010, P.R. China

Received January 11, 2022; Accepted April 8, 2022

DOI: 10.3892/mmr.2022.12727

Abstract. CD138$^+$ T cells, the majority of which are CD4 and CD8 double-negative (DN) T cells, contribute to the production of anti-dsDNA antibodies in a CD4 receptor-dependent way to promote the development of systemic lupus erythematosus (SLE). Accumulation of CD138$^+$ T cells in the spleen of MRL/*lpr* mice was significantly reduced by prednisone. Reduced expression of CD138 in DN T cells induced by prednisone treatment alleviated the accumulation of DN T cells in MRL/*lpr* mice. The frequency of CD138$^+$ cells in CD4$^+$ T cells of prednisone-treated MRL/*lpr* mice was also significantly reduced, which subsequently contributed to reduced production of anti-dsDNA antibody in the prednisone-treated MRL/*lpr* mice. Additionally, prednisone significantly reduced serum IgG and IgG subsets and simultaneously increased IgM secretion in serum. This suggested that glucocorticoids played a protective role during SLE treatment in MRL/*lpr* mice by promoting the production of IgM. The present study provides new insights into the mechanism of glucocorticoid for the treatment of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic and multisystem autoimmune disease that predominantly affects women, especially between puberty and menopause (1,2). However, the mechanisms of SLE are complex and undeciphered. Although B cells play a central role in adaptive immunity, recent studies on SLE suggest both T and B cells are involved in the progression of SLE (3-5). Fas (CD95) is a member of the tumor necrosis factor receptor family and interacts with Fas ligand (FasL) after T cell receptor (TCR) activation to initiate apoptosis (6). Fas-deficiency in MRL/*lpr* mice leads to CD4$^-$ and CD8$^-$ double-negative (DN) T cell accumulation in MRL/*lpr* mice, resulting in lymphadenectasis and splenomegaly (7,8). DN T cells have been demonstrated to play an important role in the development of SLE (3,9,10). Studies have shown that DN T cells in MRL/*lpr* mice are strongly cytotoxic (6) and overexpression of FasL on hyperactivated cytolytic DN T cells results in an autoimmune disease that attacks tissues that express low levels of the Fas receptor (6). Recent studies have also observed an accumulation of DN T cells during lupus nephritis, which induces or exacerbates tissue injury (3,11). However, the mechanism that results in the accumulation of DN T cells remains to be deciphered (12-20). Interestingly, recent studies have found that the majority of DN T cells also express CD138 in MRL/*lpr* lupus mice (21-23). Importantly, our recent study demonstrated that CD138 expression in CD3$^+$ T cells could dramatically prevent CD3$^+$ T cell apoptosis and significantly contribute to the accumulation of DN T cells (Xie T, Liu X and Li P; unpublished data).

Syndecan-1/CD138 is a marker of plasma cells in lymphocytes that are believed to originate from B cells (24,25). CD138$^+$ T cells, which express both CD3 and CD138, were identified in murine systemic lupus erythematosus (SLE) models (21-23). These abnormal CD138$^+$ cells have also been reported recently to be plasmablastic B-cell neoplasms as observed in clinical cases (26). These results indicate that CD138 could be expressed on CD3$^+$ T cells of both humans and mice. However, CD138$^+$ T cells constitute only a small fraction of cells in the spleen of non-lupus-prone mice (21,23). The majority of the CD138$^+$ T cells in MRL/*lpr* mice are also CD4 and CD8 double-negative (21-23). Previous studies have indicated that CD138$^+$ T cells play a key role in the progression of lupus in MRL/*lpr* mice. The accumulation of CD138$^+$ T cells in the spleen of MRL/*lpr* mice has been observed and progressively increase with the development of the disease (21). Studies have also demonstrated that CD138$^+$ T cells significantly contribute to the production of anti-double-stranded (ds)DNA antibodies both *in vivo* and *in vitro*, which in turn promote the development of lupus (21,27).

Female MRL/*lpr* mice have been used extensively as animal models for SLE. These mice show signs of lymphadenopathy and splenomegaly, lupus nephritis, and *in vivo* inflammation

Correspondence to: Dr Ping Li, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing Institute of Chinese Medicine, 23 Art Gallery Back Street, Dongcheng, Beijing 100010, P.R. China
E-mail: liping@bjzhongyi.com

Key words: CD138$^+$ T cells, double-negative T cells, glucocorticoid, prednisone, autoimmune, systemic lupus erythematosus

with increased levels of multiple cytokines. Furthermore, autoantibodies such as anti-dsDNA and anti-SM which are detected in SLE patients were also observed in MRL/*lpr* mouse models (21,28,29). Glucocorticoid treatment is the first-line treatment option and has shown a significant therapeutic effect for the clinical treatment of SLE (30-33). Glucocorticoids have been demonstrated to have a significant therapeutic effect for both SLE patients and SLE murine models by reducing *in vivo* autoantibody secretion, including anti-dsDNA antibodies (30-34). In the present study, we further investigated the underlying mechanism of glucocorticoid for the treatment of SLE. We investigated whether glucocorticoid could prevent $CD138^+$ T cell accumulation and suppress CD138 expression in DN T cells to alleviate DN T cells accumulation in MRL/*lpr* mice.

Materials and methods

Animals. A total of 8 female MRL/MPJ mice and 16 female MRL/*lpr* lupus mice were purchased from the Slac Laboratory (Shanghai, China). Mice were housed at 22±1˚C with a relative humidity of 50-60% with a 12-h light/dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Beijing Institute of Chinese Medicine and were performed in accordance with Animal Research protocols for reporting of *In Vivo* Experiments (ARRIVE) guidelines (35,36) and institutional regulations.

Methods. The 4-week-old female MRL/MPJ mice (25-30 g) and 4-week-old female MRL/*lpr* lupus mice (25-30 g) were acclimatized for one week. Eight female MRL/MPJ mice were used as negative controls (ddH_2O, n=8), while the remaining 16 MRL/*lpr* lupus mice were randomly divided into two groups, i.e., the vehicle group (ddH_2O, n=8) and the prednisone (PNS) 5.0 mg/kg/day group (n=8). Oral administration was performed daily from 9 to 16 weeks of age. Body weight was recorded every week during the study (Fig. 1A). At the 17-18th week of age, mice were anesthetized using 1% sodium pentobarbital (80 mg/kg) for serum collection, and then euthanized by cervical dislocation under anesthesia. The following tissues were harvested: lymph nodes and spleen (isolated and weighed), and kidneys (for histology).

Histology. To observe renal pathologic changes in lupus-prone mice, the kidneys were harvested at 17-18 weeks of age and snap-frozen in OCT compound for frozen tissue sections or fixed in 4% paraformaldehyde. Paraformaldehyde-fixed kidneys were embedded in paraffin and then sectioned at 4-μm thickness. Hematoxylin and eosin (H&E), periodic acid schiff (PAS), periodic acid-silver metheramine (PASM), and Masson trichrome staining was performed on the paraffin sections.

Measurement of total IgG, anti-dsDNA IgG, and anti-nuclear antibodies in the serum. Serum levels of total IgG, anti-dsDNA IgG, and anti-nuclear antibody (ANA) were measured using ELISA (total IgG ELISA kit, cat. no. 88-50400-22; Thermo Fisher Scientific, Inc.; anti-dsDNA IgG, cat. no. 5120 and ANA ELISA kit, cat. no. 5210; Alpha Diagnostic International), and were performed according to the manufacturers' instructions. Diluted serum samples were added onto coated 96-well plates and incubated for 1 h at room temperature. Afterward, the plates were washed with wash buffer and then incubated with anti-mouse IgG-HRP conjugate for 30 min at room temperature. Then, TMB solution was added and incubated at room temperature for 15 min. The reaction was terminated with a stop solution and the plates were read at 450 nm absorbance using a microplate reader.

Measurement of antibody isotypes in serum using the Luminex platform. Serum levels of multiple antibody subtypes were measured using the Luminex assay kits (Thermo Fisher Scientific, Inc.; cat. no. EPX070-20815-901). Measurements were performed according to the manufacturer's instructions. Diluted serum samples were added onto 96-well plates coated with magnetic beads and incubated for 120 min after vortexing. The beads were then washed, and the detection antibody mixture was added and incubated for 30 min at room temperature. After incubation and plate washing, the samples were analyzed on the Luminex™ platform (Thermo Fisher Scientific, Inc.).

Measurement of urine protein levels. Urine samples from individual 16-week-old mice were collected for 24 h. The concentration of proteins in the urine was determined using the Coomassie brilliant blue dye-binding assay kit (Biokits Tech. Inc.; cat. no. BCBU-027) and was performed according to the manufacturer's instructions (Biokits Tech. Inc.).

Flow cytometry. After harvesting the spleen, single-cell suspensions of splenocytes were obtained by filtering through a 70-μm cell strainer. Splenocytes were incubated on ice with CD16/CD32 monoclonal antibody (eBiosience/Thermo Fisher Scientific, Inc.; ready to use; cat. no. 14-0161-85) for 15 min, and then red blood cells were lysed using lysis buffer (BD Biosciences). The splenocytes were then fixed and permeabilized (Fixation/Permeabilization solution; BD Biosciences) before intracellular staining was performed. Cells were stained with the following ready to use antibodies for flow cytometry analysis: anti-CD3 PE-cy7 (eBiosience; Thermo Fisher Scientific, Inc.; cat. no. 25-0032-82), anti-CD4 FITC (eBiosience; Thermo Fisher Scientific, Inc.; cat. no. 11-0041-82), anti-CD8 APC (eBiosience; Thermo Fisher Scientific, Inc.; cat. no. 17-0081-82), anti-CD19 APC-cy7 (eBiosience; Thermo Fisher Scientific, Inc.; cat. no. 47-0193-82), anti-CD138 PE (Biolegend Inc.; cat. no. 142504), and anti-CD69 PE (Biolegend Inc.; cat. no. 104507). FACs data were analyzed using Flowjo software version 10.6 for PC (Tree Star, Inc.).

Cellular stimulation. CpGC (1 μM) or combined with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin were used to stimulate and activate the cultured splenocytes from the mice.

Statistical analysis. Data from all experiments are expressed as mean ± SD and were analyzed using SPSS software 17.0 (SPSS, Inc.). Comparisons between the groups were performed for statistical significance using Mann-Whitney U test between two groups or one-way analysis of variance followed by Tukey's

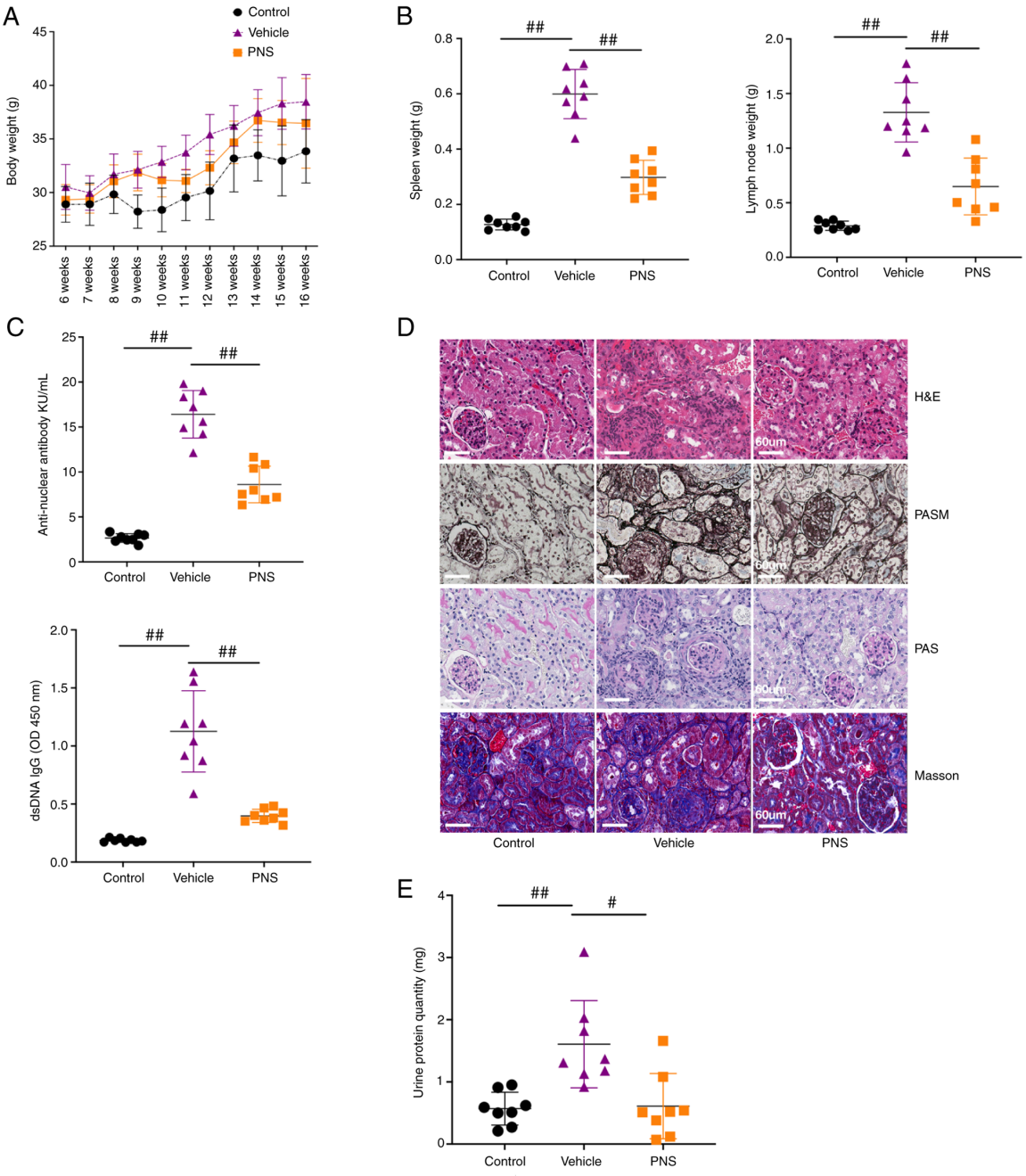

Figure 1. (A) Body weight of the mice; n=8 per group from two independent experiments. (B) Scatter plots of spleen weight, and lymph node weight; n=8 per group from two independent experiments. (C) Scatter plots showing serum ANA levels and anti-dsDNA IgG antibody levels; n=8 per group from two independent experiments. (D) Representative paraffin kidney sections stained with H&E, PASM, PAS and Masson. Samples from the three groups were used for H&E, PASM, and PAS staining and were different from the samples used to perform Masson staining; original magnification, x400; scale bars, 60 μm; n=5 per group from two independent experiments. (E) Scatter plots of protein levels in urine samples collected over 24 h from 16-week old mice; 24-h urinary protein quantity=urinary protein levels per liter x24 h urinary volume; n=8 per group from two independent experiments; Data are presented as mean ± SD; $^{\#}$P<0.05, $^{\#\#}$P<0.01, by one-way analysis of variance, vs. the vehicle-treated mice. ANS, anti-nuclear antibody; anti-dsDNA, anti-double-stranded DNA; PNS, prednisone; H&E, hematoxylin and eosin; PAS, periodic acid Schiff; PASM, periodic acid-silver metheramine.

post hoc test for multiple group comparisons. Differences with P-values <0.05 were considered statistically significant.

Results

Successful construction of the murine SLE model and the significant therapeutic effects of prednisone. Compared to the control mice, significant enlargement of the spleen and lymph nodes, increased serum levels of anti-nuclear antibody (ANA) and anti-dsDNA IgG antibodies, and elevated urine protein levels in our murine lupus model were observed (Fig. 1B, C and E). Additionally, obvious renal injuries in the vehicle-treated lupus mice were observed and were characterized by hyaline deposits, interstitial and perivascular cellular inflammation infiltration, cellular crescent formation, glomerular fibrosis, glomerulosclerosis, and tubular cell necrosis (Fig. 1D). These results indicate that the murine lupus model was successfully constructed. As expected,

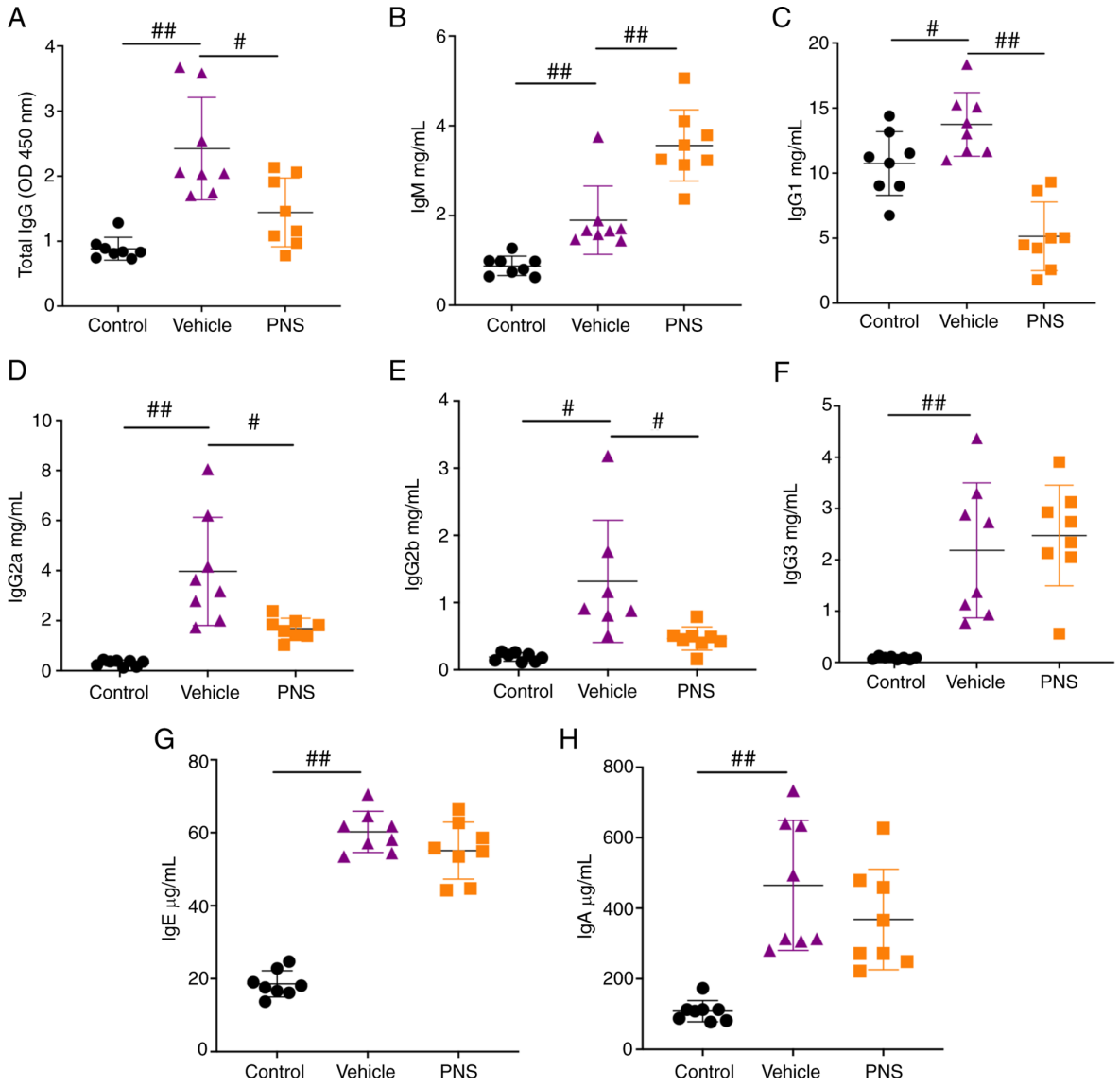

Figure 2. (A) Scatter plots showing serum levels of total IgG antibodies. (B) Scatter plots showing serum levels of IgM antibodies. (C) Scatter plots showing serum levels of IgG1 antibodies. (D) Scatter plots showing serum levels of IgG2a antibodies. (E) Scatter plots showing serum levels of IgG2b antibodies. (F) Scatter plots showing serum levels of IgG3 antibodies. (G) Scatter plots showing serum levels of IgE antibodies. (H) Scatter plots showing serum levels of IgA antibodies; Data are presented as mean ± SD; n=7-8 per group from two independent experiments; $^{\#}$P<0.05, $^{\#\#}$P<0.01 by one-way analysis of variance, vs. the vehicle-treated mice. PNS, prednisone.

prednisone (PNS) treatment exhibited significant therapeutic effects on lupus mice by significantly alleviating the enlargement of the spleen and lymph nodes, decreasing serum levels of ANA and anti-dsDNA IgG antibodies, reducing urine protein levels, and improving histopathological injuries in the renal tissues (Fig. 1).

Prednisone reduces total IgG, IgG1, and IgG2a levels in the serum of the lupus mice, while simultaneously increasing IgM levels. We found that the levels of total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, IgE, and IgA in the serum were significantly increased in lupus mice compared to the control mice (Fig. 2A-H). However, the levels of total IgG, IgG1, IgG2a, and IgG2b were significantly reduced in the MRL/*lpr* mice treated with PNS (Fig. 2A and C-E). In addition, we observed a further increase in IgM levels in the serum of MRL/*lpr* mice (Fig. 2B). Our results indicate that PNS reduced the production of total IgG, IgG1, IgG2a, and IgG2b in the serum of the MRL/*lpr* mice, and simultaneously increased serum IgM levels in the MRL/*lpr* mice.

Prednisone prevents activation of CD3^{+} T cells in the MRL/lpr mice. We stimulated and activated splenocytes using PMA and ionomycin for 5 h. Activated T lymphocytes were stained with CD69 (34,37). Compared to the vehicle-treated MRL/*lpr* mice, T cells and T cell subsets including CD4^{+}, CD8^{+} and DN T cells in the MRL/MPJ mice had significantly higher frequencies of CD69^{+} cells after 5 h of stimulation (Fig. 3A-C and E), suggesting that T cells and their subsets in MRL/MPJ mice were more easily activated. Consistent with these results, we observed that both CD4^{+} and CD8^{+} T cell frequencies in splenocytes of MRL/MPJ and MRL/*lpr* mice were equal before *in vitro* stimulation (Fig. 3D). But both CD4^{+} and CD8^{+} T cell frequencies in splenocytes of the MRL/MPJ mice were significantly higher

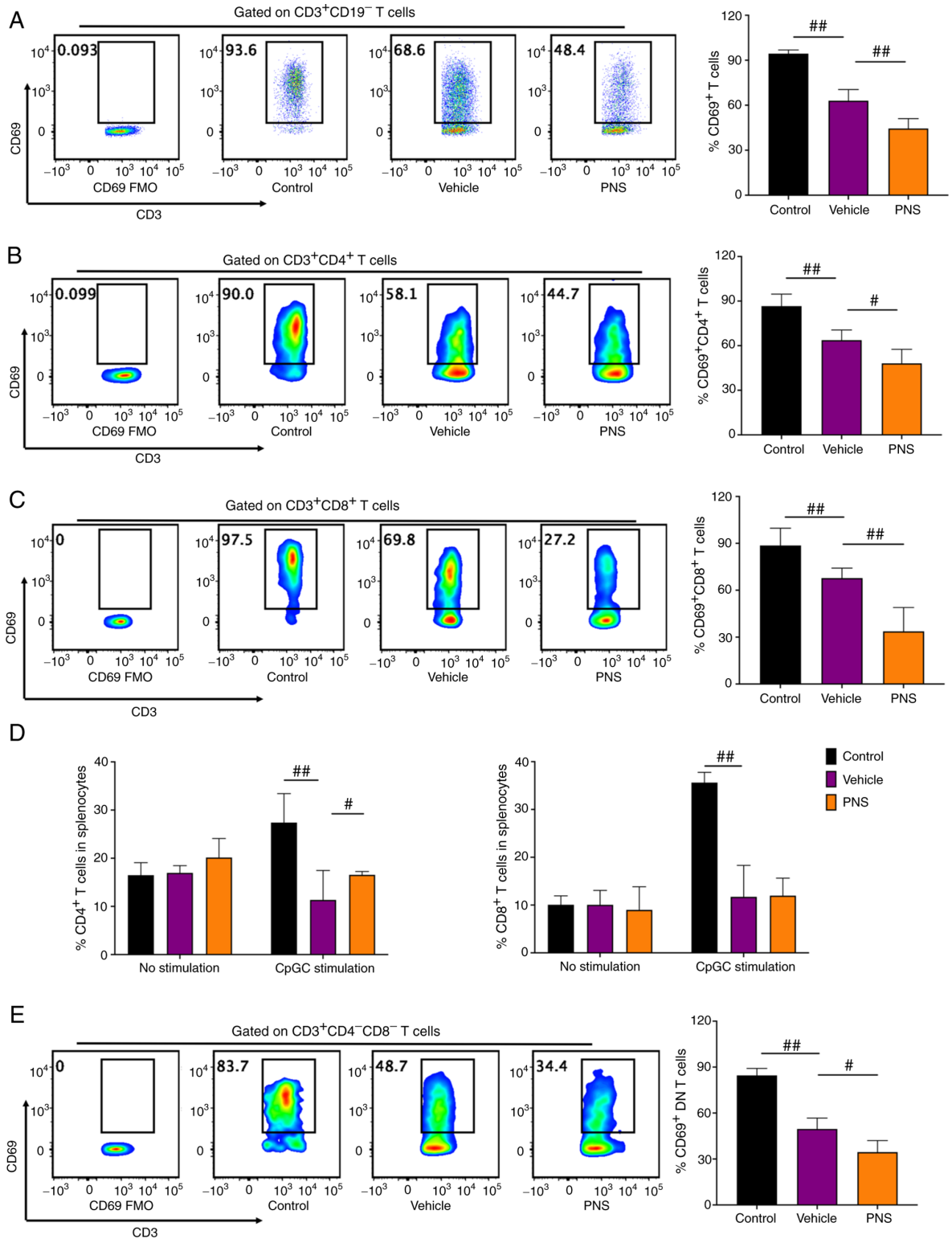

Figure 3. (A) Representative flow cytometry analyses and bar charts denote the frequency of CD69+ cells in CD3+ T cells in stimulated splenocytes. (B) Representative flow cytometry analyses and bar charts denote the frequency of CD69+ cells in CD4+ T cells in stimulated splenocytes. (C) Representative flow cytometry analyses and bar charts denote the frequency of CD69+ cells in CD8+ T cells in stimulated splenocytes. (D) Bar charts showing the frequency of CD4+ and CD8+ T cells before and after 24 h of CpGC stimulation in splenocytes. (E) Representative flow cytometry analyses and bar charts denote the frequency of CD69+ cells in double-negative (DN) T cells in stimulated splenocytes; Data are presented as mean ± SD; n=4-5 per group from two independent experiments; #P<0.05, ##P<0.01 by one-way analysis of variance, vs. the vehicle-treated mice. PNS, prednisone.

than the frequencies in splenocytes of the MRL/*lpr* mice after 24 h of *in vitro* CpGC stimulation of splenocytes (Fig. 3D).

PNS showed significant effects on activation of T cells and T cell subsets. PNS significantly decreased CD69+ cell frequencies in CD3+ T cells and its subsets including CD4+, CD8+ and DN T cells in splenocytes of the MRL/*lpr* mice (Fig. 3A-C and E). The decrease in CD69+ cell frequency in CD8+ T cells of the PNS-treated mice was more significant

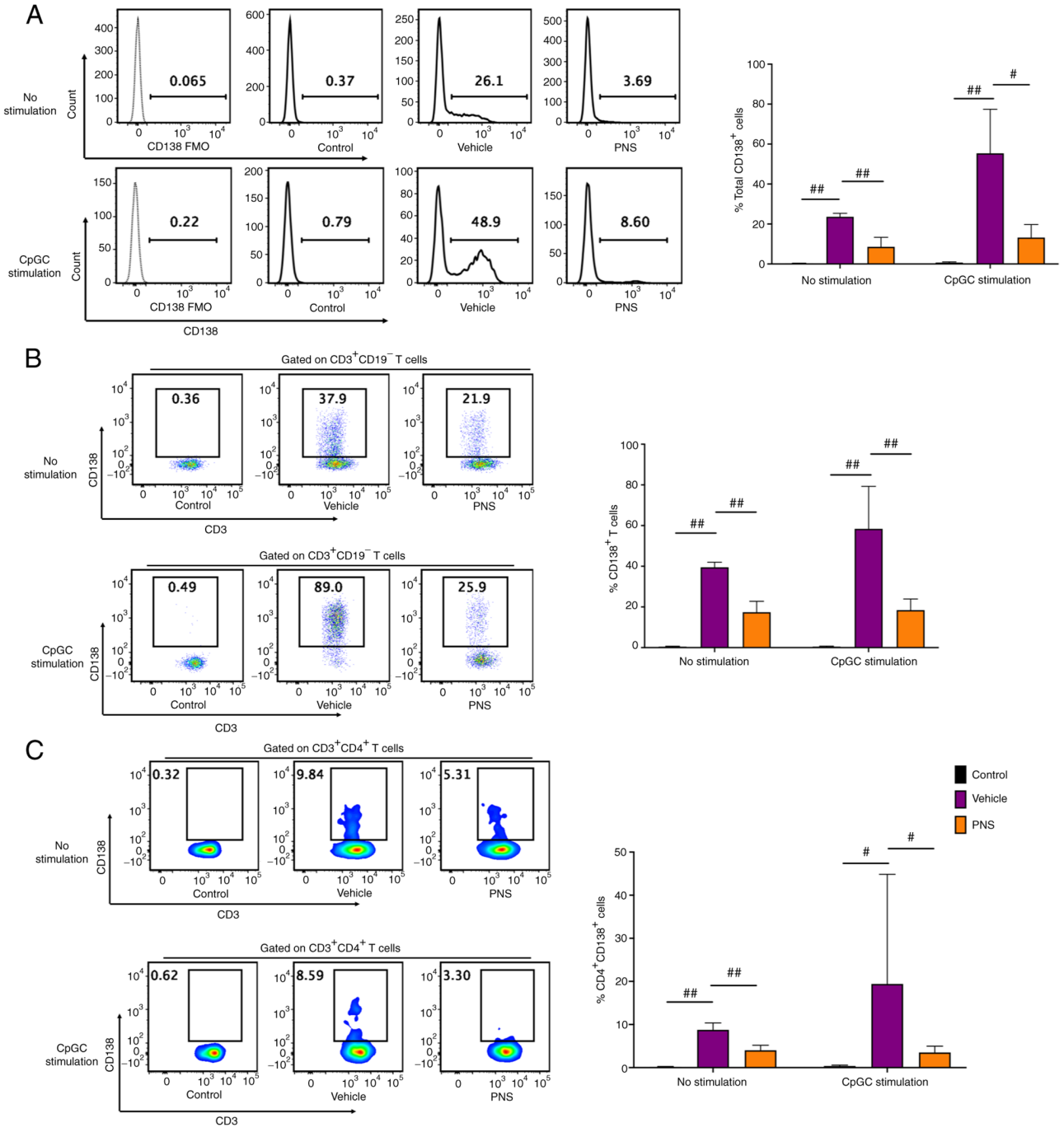

Figure 4. (A) Representative flow cytometry analyses and bar charts denote the frequency of CD138⁺ cells in splenocytes before and after splenocytes were CpGC stimulated for 24 h. (B) Representative flow cytometry analyses and bar charts denote the frequency of CD138⁺ T cells in CD3⁺ T cells before and after splenocytes were CpGC stimulated for 24 h. (C) Representative flow cytometry analyses and bar charts denote the frequency of CD138⁺ cells in CD4⁺ T cells before and after 24 h of CpGC stimulation. All data are presented as mean ± SD; n=4 per group from two independent experiments; $^{\#}P<0.05$, $^{\#\#}P<0.01$ by Mann-Whitney U or one-way analysis of variance test. PNS, prednisone.

compared with that in the CD4⁺ and DN T cells. However, PNS did not significantly reduce CD8⁺ T cell frequency in the splenocytes of the MRL/*lpr* mice (Fig. 3D). Contrarily, CD4⁺ T cell frequency in the splenocytes of the MRL/*lpr* mice with PNS treatment was significantly increased (Fig. 3D).

Prednisone prevents CD138 expression in T cells of MRL/lpr mice. Isolated splenocytes from the MRL/MPJ mice without stimulation had a near absence of CD138⁺ cells, whereas the splenocytes of Fas-deficiency MRL/*lpr* mice had a significant increase in CD138⁺ cell frequency compared with those of the MRL/MPJ mice (Fig. 4A). We next co-cultured and stimulated splenocytes with CpGC for 24 h. We also observed that CD138⁺ cells were accumulated in splenocytes of the MRL/*lpr* mice but not in the MRL/MPJ mice (Fig. 4A). Compared to the vehicle-treated lupus mice, the CD138⁺ cell frequencies in splenocytes of the PNS-treated lupus mice were significantly reduced both with and without 24 h of CpGC stimulation (Fig. 4A).

The frequencies of CD138⁺ cells in CD3⁺ T cells of the MRL/MPJ mice were still negligible; however, CD138 was

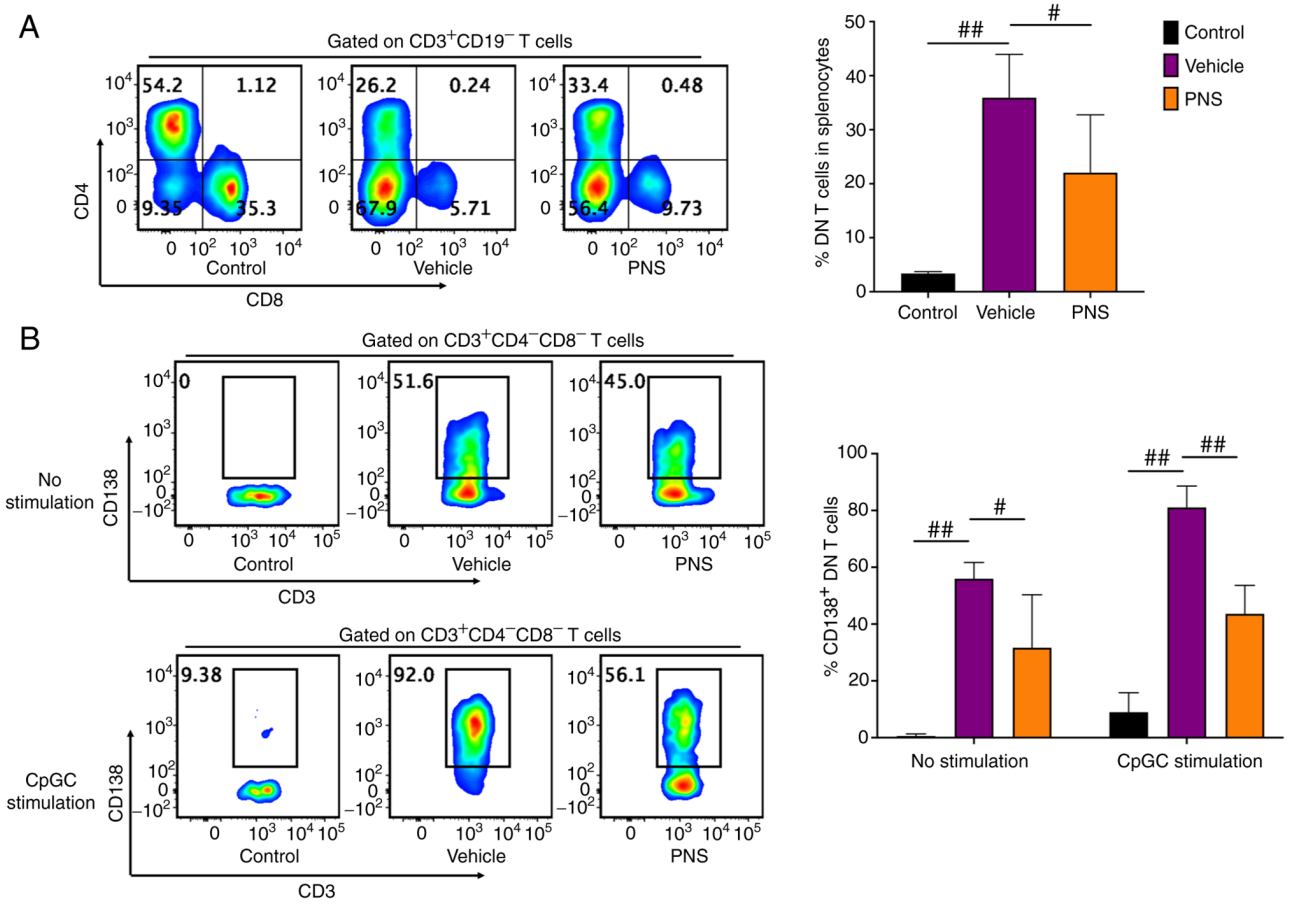

Figure 5. (A) Representative flow cytometry analyses showing double-negative (DN) T cell accumulation in T cells of MRL/*lpr* mice and bar charts denote the frequency of DN T cells in the splenocytes of mice. (B) Representative flow cytometry analyses and bar charts showing the frequency of DN T cells in the splenocytes of mice. All data are presented as mean ± SD; n=4-5 per group from two independent experiments; $^{\#}$P<0.05, $^{\#\#}$P<0.01, by Mann-Whitney U or one-way analysis of variance test. PNS, prednisone.

abundantly expressed on CD3$^+$ T cells in the MRL/*lpr* mice (Fig. 4B) both before and after 24 h of CpGC stimulation of splenocytes *in vitro*. CD138$^+$ T cell frequencies in CD3$^+$ T cells of the MRL/*lpr* mice with oral administration of PNS were significantly decreased compared to the vehicle-treated MRL/*lpr* mice both before and after 24 h of CpGC stimulation of splenocytes *in vitro* (Fig. 4B).

In addition, we observed that CD138 was also expressed in the CD4$^+$ T cells in the MRL/*lpr* mice but not in the MRL/MPJ mice both with and without 24 h of CpGC stimulation of splenocytes *in vitro* (Fig. 4C). PNS also significantly prevented CD138 expression in CD4$^+$ T cells. The CD138$^+$ cell frequencies in the CD4$^+$ T cells were significantly decreased in the MRL/*lpr* mice after prednisone treatment compared to the vehicle-treated mice both before and after CpGC stimulation of splenocytes *in vitro* (Fig. 4C).

Prednisone prevents DN T cell accumulation and CD138 expression in DN T cells of MRL/lpr mice. DN T cells strikingly accumulated in the splenocytes of the Fas-deficiency MRL/*lpr* mice but not in the MRL/MPJ mice (Fig. 5A). PNS significantly relieved DN T cell accumulation in the splenocytes of the MRL/*lpr* mice (Fig. 5A). Importantly, DN T cells in the MRL/*lpr* mice but not in the MRL/MPJ mice commonly expressed CD138 (Fig. 5B). However, PNS also prevented CD138 expression in DN T cells of the MRL/*lpr* mice. The frequency of CD138$^+$ cells in DN T cells of the PNS-treated lupus mice was significantly reduced compared to the vehicle-treated mice (Fig. 5B). Furthermore, even after 24 h of CpGC stimulation of splenocytes *in vitro*, the CD138$^+$ cell frequency in DN T cells of the PNS-treated MRL/*lpr* mice also showed a significant reduction compared to the vehicle-treated mice (Fig. 5B).

Discussion

The present study results demonstrated that prednisone (PNS) significantly relieved systemic lupus erythematosus (SLE) symptoms in MRL/*lpr* mice by alleviating enlargement of the spleen and lymph nodes, reducing the production of autoantibody in the serum including anti-double-stranded (anti-ds) DNA antibodies IgG antibody and anti-nuclear antibody (ANA), and ameliorating renal tissue injury and simultaneously preventing the accumulation of double negative (DN) T cells in splenocytes of MRL/*lpr* mice. In addition, it was demonstrated that PNS had a significant effect on CD138 expression in CD3$^+$ T cells of the MRL/*lpr* mice. PNS prevented CD138$^+$ T cell accumulation in the MRL/*lpr* mice and inhibited CD138 expression in both CD4$^+$ and DN T cells of the MRL/*lpr* mice. In addition, PNS played a protective role

by significantly increasing levels of IgM secretion in the serum of the MRL/*lpr* mice. The results showed new insights into the mechanisms of glucocorticoid on SLE treatment.

PNS, a glucocorticoid drug, exhibited significant therapeutic effects on MRL/*lpr* lupus mice in our study. We observed that PNS was able to significantly reduce the production of total IgG and multiple IgG antibody subsets such as IgG1, IgG2a, and IgG2b, and simultaneously increased IgM production in the serum of the MRL/*lpr* mice. Previous studies have demonstrated that in humans, IgA deficiency is associated with autoimmunity. However, this differs between SLE humans and SLE mice. IgM antibody has been demonstrated to be a protective antibody isotype in MRL/*lpr* mice (38-41). IgM deficiency was previously found to significantly contribute to accelerated development of lupus and elevated levels of IgG autoantibody secretion in MRL/*lpr* mice (38-41). This indicated that PNS could ameliorate lupus in MRL/*lpr* mice by increasing the production of IgM. This suggests that glucocorticoid ameliorates SLE by increasing the production of protective antibody subsets. Furthermore, PNS significantly decreased autoantibody production, including anti-dsDNA antibody in the MRL/lpr mice. Our results also showed that PNS significantly reduced $CD4^+CD138^+$ T cell frequency in $CD4^+$ T cells of the MRL/*lpr* mice. A previous study demonstrated that $CD138^+$ T cells contributed to the production of anti-dsDNA antibodies both *in vivo* and *in vitro* by a CD4 receptor-dependent mechanism. This suggested that $CD4^+CD138^+$ T cells were the autoreactive $CD4^+$ T cells that promoted anti-dsDNA antibody production (21). This indicated that the decline in CD138 expression in $CD4^+$ T cells induced by PNS prevented anti-dsDNA antibody production, which resulted in PNS decreasing anti-dsDNA autoantibody levels in the serum.

DN T cells have been shown to accumulate in the peripheral blood of SLE patients and the spleen of Fas-deficiency lupus mice (3,9,10). Our results showed that PNS significantly prevented DN T cell accumulation in splenocytes of the MRL/*lpr* mice. Previous research has demonstrated that DN T cells are involved in the development of systemic inflammation and tissue damage in lupus patients (3). Recent research has demonstrated that the adoptive transfer of DN T cells aggravates the pathology in young lupus mice, while significant infiltration of DN T cells was observed in both adult and pediatric lupus kidneys (3). However, our results showed that the majority of DN T cells in splenocytes of the MRL/*lpr* mice were CD138 positive. Previous research also showed that $CD138^+$ T cells were associated with the production of anti-dsDNA antibodies both *in vivo* and *in vitro*, and demonstrated adoptive transfer of $CD138^+$ T cells significantly contributed to renal tissue injuries in MRL/*lpr* lupus mice (21). These results demonstrated that $CD138^+$ T cells are also strongly associated with the progression of lupus in MRL/*lpr* mice. Our recent study demonstrated that CD138 expression in $CD3^+$ T cells strikingly prevented the apoptosis of $CD3^+$ T cells and strikingly contributed to the accumulation of DN T cells in T cells of MRL/*lpr* mice (Xie T, Liu X and Li P; unpublished data). In the present study, our results also showed that DN T cells in the MRL/*lpr* mice had a high level of CD138 expression. PNS treatment significantly decreased $CD138^+$ cell frequency in DN T cells of lupus mice. Our results demonstrated that reduced frequency of $CD138^+$ cells in DN T cells contributed to reducing the accumulation of DN T cells in MRL/*lpr* mice after PNS administration. However, information and data regarding CD138 expression in T cells in MRL/*lpr* mice is still limited. The specific mechanism and signaling pathways involved in CD138 expression in $CD3^+$ T cells of MRL/*lpr* mice are yet to be deciphered and have not been consistent between studies. Hence, more studies and data are needed to demonstrate the underlying mechanism and signaling pathways in the glucocorticoid-mediated prevention of CD138 expression in T cells of MRL/*lpr* mice.

CpG DNA, which includes CpGA, CpGB, and CpGC, are Toll-like receptor (TLR) agonists (42) that activate and induce interferon (IFN)-α secretion in plasmacytoid dendritic cells (pDCs) (43). CpGC stimulation was used in this study to mimic *in vivo* conditions in SLE patients (44), i.e., to induce the secretion of IFN-α to promote SLE development and aggravate tissue injury (43,45). Our present study showed that the frequencies of $CD4^+$ and $CD8^+$ T cells in fresh splenocytes of the control mice were nearly equal to the frequencies in the MRL/*lpr* mice. However, when splenocytes were stimulated by CpGC, $CD4^+$ and $CD8^+$ T cell frequencies in the control mice were significantly higher than the frequencies in the Fas-deficiency MRL/*lpr* mice. Furthermore, T cells in the control mice including $CD4^+$ T cells and $CD8^+$ T cells, were more likely to be activated in response to T cell stimulation compared to lupus mice. Our results indicated that both $CD4^+$ and $CD8^+$ T cells in the Fas-deficiency MRL/*lpr* mice had defective proliferation and activation.

In summary, PNS had a significant therapeutic effect on MRL/*lpr* lupus mice. PNS significantly prevented $CD138^+$ T cell accumulation in the MRL/*lpr* mice. CD138 expression in DN T cells of the MRL/*lpr* mice was inhibited by PNS which contributed to PNS alleviating DN T cell accumulation. PNS also prevented CD138 expression in $CD4^+$ T cells which significantly resulted in reduced anti-dsDNA antibody production in MRL/*lpr* mice after PNS treatment. In addition, PNS also decreased IgG and IgG subset production and simultaneously promoted IgM secretion which plays a preventive role in the progression of SLE treatment. Our results provide new insights into the therapeutic effects and mechanisms of glucocorticoid treatment for SLE.

Acknowledgements

The authors would like to acknowledge Dr Sabry Hamza (Tessa Therapeutics Ltd., Singapore) for editing this manuscript.

Funding

This study was funded by the Beijing Postdoctoral Research Foundation (grant no. ZZ2019-23) and the MiaoPu Research Foundation of the Beijing Institute of Chinese Medicine (grant no. MP-2020-45).

Availability of data and materials

The data generated in the present study are available from the corresponding author on reasonable request.

Authors' contributions

TX conceived the study and wrote the manuscript. TX and PL designed the experiments. TX and HL performed the laboratory work. TX and HL performed the data analysis. PL revised and edited the manuscript. TX and PL confirm the authenticity of all the raw data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work (including the data presented) are appropriately investigated and resolved.

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Beijing Institute of Chinese Medicine and were performed in accordance with Animal Research protocols for reporting of *In Vivo* Experiments (ARRIVE) guidelines and institutional regulations.

Patient consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

References

1. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, Ruiz-Irastorza G and Hughes G: Systemic lupus erythematosus. Nat Rev Dis Primers 2: 16039, 2016.
2. Dörner T and Furie R: Novel paradigms in systemic lupus erythematosus. Lancet 393: 2344-2358, 2019.
3. Alexander JJ, Jacob A, Chang A, Quigg RJ and Jarvis JN: Double negative T cells, a potential biomarker for systemic lupus erythematosus. Precis Clin Med 3: 34-43, 2020.
4. Chesnutt MS, Finck BK, Killeen N, Connolly MK, Goodman H and Wofsy D: Enhanced lymphoproliferation and diminished autoimmunity in CD4-deficient MRL/lpr mice. Clin Immunol Immunopathol 87: 23-32, 1998.
5. Nagasu A, Mukai T, Iseki M, Kawahara K, Tsuji S, Nagasu H, Ueki Y, Ishihara K, Kashihara N and Morita Y: Sh3bp2 Gain-Of-function mutation ameliorates lupus phenotypes in B6.MRL-Fas^{lpr} Mice. Cells 8: 402, 2019.
6. Benihoud K, Bonardelle D, Bobé P and Kiger N: MRL/lpr CD4-CD8$^-$ and CD8$^+$ T cells, respectively, mediate Fas-dependent and perforin cytotoxic pathways. Eur J Immunol 27: 415-420, 1997.
7. Martina MN, Noel S, Saxena A, Rabb H and Hamad AR: Double negative (DN) αβ T cells: Misperception and overdue recognition. Immunol Cell Biol 93: 305-310, 2015.
8. Corneth OBJ, Schaper F, Luk F, Asmawidjaja PS, Mus AMC, Horst G, Heeringa P, Hendriks RW, Westra J and Lubberts E: Lack of IL-17 Receptor A signaling aggravates lymphoproliferation in C57BL/6 lpr mice. Sci Rep 9: 4032, 2019.
9. Shaltout AS, Sayed D, Badary MS, Nafee AM, El Zohri MH, Bakry R and Ahmed SH: Effect of IL6 and IL23 on double negative T cells and anti ds-DNA in systemic lupus erythematosus patients. Hum Immunol 77: 937-943 2016.
10. Brandt D and Hedrich CM: TCRαβ$^+$CD3$^+$CD4$^-$CD8$^-$ (double negative) T cells in autoimmunity. Autoimmun Rev 17: 422-430, 2018.
11. Getachew Y, Cusimano FA, James LP and Thiele DL: The role of intrahepatic CD3$^+$/CD4$^-$/CD8-double negative T (DN T) cells in enhanced acetaminophen toxicity. Toxicol Appl Pharmacol 280: 264-271, 2014.
12. Hedrich CM, Rauen T, Crispin JC, Koga T, Ioannidis C, Zajdel M, Kyttaris VC and Tsokos GC: cAMP-responsive element modulator α (CREMα) trans-represses the transmembrane glycoprotein CD8 and contributes to the generation of CD3+CD4-CD8-T cells in health and disease. J Biol Chem 288: 31880-31887, 2013.
13. Hedrich CM, Crispín JC, Rauen T, Ioannidis C, Koga T, Rodriguez Rodriguez N, Apostolidis SA, Kyttaris VC and Tsokos GC: cAMP responsive element modulator (CREM) α mediates chromatin remodeling of CD8 during the generation of CD3+CD4-CD8-T cells. J Biol Chem 289: 2361-2370, 2014.
14. Merino R, Fossati L, Iwamoto M, Takahashi S, Lemoine R, Ibnou-Zekri N, Pugliatti L, Merino J and Izui S: Effect of long-term anti-CD4 or anti-CD8 treatment on the development of lpr CD4-CD8-double negative T cells and of the autoimmune syndrome in MRL-lpr/lpr mice. J Autoimmun 8: 33-45, 1995.
15. Ohteki T, Iwamoto M, Izui S and MacDonald HR: Reduced development of CD4-8-B220+ T cells but normal autoantibody production in lpr/lpr mice lacking major histocompatibility complex class I molecules. Eur J Immunol 25: 37-41, 1995.
16. Trimble LA, Prince KA, Pestano GA, Daley J and Cantor H: Fas-dependent elimination of nonselected CD8 cells and lpr disease. J Immunol 168: 4960-4967, 2002.
17. Koh DR, Ho A, Rahemtulla A, Fung-Leung WP, Griesser H and Mak TW: Murine lupus in MRL/lpr mice lacking CD4 or CD8 T cells. Eur J Immunol 25: 2558-2562, 1995.
18. Ezine S, Lucas B, Vicari A, Dautigny N, Vasseur F and Penit C: A novel CD45RA$^+$CD4$^+$ transient thymic subpopulation in MRL-lpr/lpr mice: Its relation to non-proliferating CD4-CD8-CD45RA$^+$ tumor cells. Int Immunol 5: 89-96, 1993.
19. Grishkan IV, Ntranos A, Calabresi PA and Gocke AR: Helper T cells down-regulate CD4 expression upon chronic stimulation giving rise to double-negative T cells. Cell Immunol 284: 68-74, 2013.
20. Zhang D, Yang W, Degauque N, Tian Y, Mikita A and Zheng XX: New differentiation pathway for double-negative regulatory T cells that regulates the magnitude of immune responses. Blood 109: 4071-4079, 2007.
21. Liu L, Takeda K and Akkoyunlu M: Disease stage-specific pathogenicity of CD138 (Syndecan 1)-expressing T cells in systemic lupus erythematosus. Front Immunol 11: 1569, 2020.
22. Seagal J, Leider N, Wildbaum G, Karin N and Melamed D: Increased plasma cell frequency and accumulation of abnormal syndecan-1plus T-cells in Igmu-deficient/lpr mice. Int Immunol 15: 1045-1052, 2003.
23. Mohamood AS, Bargatze D, Xiao Z, Jie C, Yagita H, Ruben D, Watson J, Chakravarti S, Schneck JP and Hamad AR: Fas-mediated apoptosis regulates the composition of peripheral alphabeta T Cell repertoire by constitutively purging out double negative T cells. PLoS One 3: e3465, 2008.
24. Lu LD, Stump KL, Wallace NH, Dobrzanski P, Serdikoff C, Gingrich DE, Dugan BJ, Angeles TS, Albom MS, Mason JL, *et al*: Depletion of autoreactive plasma cells and treatment of lupus nephritis in mice using CEP-33779, a novel, orally active, selective inhibitor of JAK2. J Immunol 187: 3840-3853, 2011.
25. Calame KL: Plasma cells: Finding new light at the end of B cell development. Nat Immunol 2: 1103-1108, 2001.
26. Pan Z, Chen M, Zhang Q, Wang E, Yin L, Xu Y, Huang Q, Yuan Y, Zhang X, Zheng G and Yuan J: CD3-positive plasmablastic B-cell neoplasms: A diagnostic pitfall. Mod Pathol 31: 718-731, 2018.
27. Tsokos GC, Lo MS, Costa Reis P and Sullivan KE: New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol 12: 716-730, 2016.
28. Eisenberg RA, Craven SY, Warren RW and Cohen PL: Stochastic control of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice. J Clin Invest 80: 691-697, 1987.
29. Eisenberg RA, Craven SY and Cohen PL: Isotype progression and clonality of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice. J Immunol 139: 728-733, 1987.
30. Pinheiro SVB, Dias RF, Fabiano RCG, Araujo SA and Silva ACSE: Pediatric lupus nephritis. J Bras Nefrol 41: 252-265, 2018 (In English, Portuguese).
31. Wilhelmus S, Bajema IM, Bertsias GK, Boumpas DT, Gordon C, Lightstone L, Tesar V and Jayne DR: Lupus nephritis management guidelines compared. Nephrol Dial Transplant 31: 904-913, 2016.
32. Mok CC, Yap DY, Navarra SV, Liu ZH, Zhao MH, Lu L, Takeuchi T, Avihingsanon Y, Yu XQ, Lapid EA, *et al*: Overview of lupus nephritis management guidelines and perspective from Asia. Int J Rheum Dis 16: 625-636, 2013.
33. Dammacco R: Systemic lupus erythematosus and ocular involvement: An overview. Clin Exp Med 18: 135-149, 2018.

34. Wu Y, He S, Bai B, Zhang L, Xue L, Lin Z, Yang X, Zhu F, He P, Tang W and Zuo J: Therapeutic effects of the artemisinin analog SM934 on lupus-prone MRL/lpr mice via inhibition of TLR-triggered B-cell activation and plasma cell formation. Cell Mol Immunol 13: 379-390, 2016.
35. Kilkenny C, Browne W, Cuthill IC, Emerson M and Altman DG; NC3Rs Reporting Guidelines Working Group: Animal research: Reporting in vivo experiments: The ARRIVE guidelines. Br J Pharmacol 160: 1577-1579, 2010.
36. Zhang P, Su L, Ma F, Ji X, Su Y, Yue Q, Zhao C, Zhang S, Sun X and Zhao L: Weilan gum oligosaccharide ameliorates dextran sulfate sodium-induced experimental ulcerative colitis. Mol Med Rep 25: 52, 2022.
37. Tilstra JS, Avery L, Menk AV, Gordon RA, Smita S, Kane LP, Chikina M, Delgoffe GM and Shlomchik MJ: Kidney-infiltrating T cells in murine lupus nephritis are metabolically and functionally exhausted. J Clin Invest 128: 4884-4879, 2018.
38. Ehrenstein MR, Cook HT and Neuberger MS: Deficiency in serum immunoglobulin (Ig)M predisposes to development of IgG autoantibodies. J Exp Med 191: 1253-1258, 2000.
39. Carroll MC: A protective role for innate immunity in systemic lupus erythematosus. Nat Rev Immunol 4: 825-831, 2004.
40. Yoshizawa Y, Honda S and Shibuya A: Involvement of Fcα/μR (CD351) in autoantibody production. Mol Immunol 57: 216-219, 2014.
41. Boes M, Schmidt T, Linkemann K, Beaudette BC, Marshak-Rothstein A and Chen J: Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. Proc Natl Acad Sci USA 97: 1184-1189, 2000.
42. Zhang X, Deriaud E, Jiao X, Braun D, Leclerc C and Lo-Man R: Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. J Exp Med 204: 1107-1118, 2007.
43. Menon M, Blair PA, Isenberg DA and Mauri C: A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells Is aberrant in systemic lupus erythematosus. Immunity 44: 683-697, 2016.
44. Wu HM, Wang J, Zhang B, Fang L, Xu K and Liu RY: CpG-ODN promotes phagocytosis and autophagy through JNK/P38 signal pathway in Staphylococcus aureus-stimulated macrophage. Life Sci 161: 51-59, 2016.
45. Akiyama C, Tsumiyama K, Uchimura C, Honda E, Miyazaki Y, Sakurai K, Miura Y, Hashiramoto A, Felsher DW and Shiozawa S: Conditional upregulation of IFN-α alone Is sufficient to induce systemic lupus erythematosus. J Immunol 203: 835-843, 2019.