Chin J Integr Med 2019 Dec;25(12):902-910

Chinese Journal of Integrative Medicine

Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
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Original Article

Panaxdiol Saponins Component Promotes Hematopoiesis and Modulates T Lymphocyte Dysregulation in Aplastic Anemia Model Mice*

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ABSTRACT **Objective**: To investigate the potential efficacy of panaxadiol saponins component (PDS-C) in the treatment of aplastic anemia (AA) model mice. **Methods**: Totally 70 mice were divided into 7 groups as follows: normal, model, low-, medium-, high-dose PDS-C (20, 40, 80 mg/kg, namely L-, M-, H-PDS-C), cyclosporine (40 mg/kg), and andriol (25 mg/kg) groups, respectively. An immune-mediated AA mouse model was established in BALB/c mice by exposing to 5.0 Gy total body irradiation at 1.0 Gy/min, and injecting with lymphocytes from DBA mice. On day 4 after establishment of AA model, all drugs were intragastrically administered daily for 15 days, respectively, while the mice in the normal and model groups were administered with saline solution. After treatment, the peripheral blood counts, bone marrow pathological examination, colony forming assay of bone marrow culture, T lymphocyte subpopulation analysis, as well as T-bet, GATA-3 and FoxP3 proteins were detected by flow cytometry and Western blot. **Results**: The peripheral blood of white blood cell (WBC), platelet, neutrophil counts and hemoglobin (Hb) concentration were significantly decreased in the model group compared with the normal group (all $P<0.01$). In response to 3 dose PDS-C treatment, the WBC, platelet, neutrophil counts were significantly increased at a dose-dependent manner compared with the model group (all $P<0.01$). The myelosuppression status of AA was significantly reduced in M-, H-PDS-C groups, and hematopoietic cell quantity of bone marrow was more abundant than the model group. The colony numbers of myeloid, erythroid and megakaryocytic progenitor cells in the model group were less than those of the normal mice in bone marrow culture, while, PDS-C therapy enhanced proliferation of hematopoietic progenitor cells by significantly increasing colony numbers (all $P<0.01$). Furthermore, PDS-C therapy increased peripheral blood $CD3^+$ and $CD3^+CD4^+$ cells and reduced $CD3^+CD8^+$ cells ($P<0.05$ or $P<0.01$). Meanwhile, PDS-C treatment at medium- and high doses groups also increased $CD4^+CD25^+FoxP3^+$ cells, downregulated T-bet protein expression, and upregulated GATA-3 and FoxP3 protein expressions in spleen cells ($P<0.05$). **Conclusion**: PDS-C possesses dual activities, promoting proliferation hematopoietic progenitor cells and modulating T lymphocyte immune functions in the treatment of AA model mice.

KEYWORDS Panaxadiol saponins component, aplastic anemia, hematopoiesis, T lymphocyte

Aplastic anemia (AA) is a hematological disorder which causes pancytopenia (hemocytopenia). The patients often present with tiredness, infection and/or bleeding. At present, AA is usually treated by immunosuppressive agents and sometimes androgen.[1,2] However, the treatment response has not been always satisfactory and these drugs often cause serious side effects. A less toxic and more effective treatment becomes more desired.

The total saponins of *Panax ginseng* (TSPG) have been extracted and isolated from Chinese ginseng herb since 1992.[3-7] Our previous studies showed that TSPG stimulated the proliferation and differentiation of hematopoietic progenitor/stem cells. TSPG also has growth factor-like activity and synergistic effect

*Supported by the National Natural Science Foundation of China (No. 81774068), Medical and Health Key Project of Zhejiang Province (No. 2011ZDA021), Zhejiang Provincial Natural Science Foundation of China (No. LY14H280004)

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DOI: https://doi.org/10.1007/s11655-019-3049-z

with hematopoietic growth factors. TSPG has been developed a hospital preparation named Sheng-xue-ling Capsule (生血灵胶囊), which was approved for clinical application by Health Bureau of Zhejiang Province in 1999. During its 18 years of clinical application in treating patients with pancytopenic disorders such as chronic AA, primary immune thrombocytopenia (ITP), and chronic neutropenia, Sheng-xue-ling Capsule has shown to be clinically effective with few obvious side effects.[8-10]

Our research team has identified a biological active compounds isolated from TSPG, and termed as panaxadiol saponins component (PDS-C). In this study, we observed the potential effect of PDS-C in the treatment with immune-mediated AA in mice model.[8]

METHODS

Preparation of PDS-C Dry Powder

Total saponins of ginsenosides were extracted from *Panax ginseng* C. A. Mey., by application biological activity assays of hematopoiesis. The active components of PDS-C were isolated from TSPG by the First Affiliated Hospital of Zhejiang Chinese Medical University. In this study, PDS-C dry powder was dissolved in saline for the treatment of AA mice. The composition of PDS-C was identified by high performance liquid spectrometry (HPLC) using specific ginsenoside monomers as reference standards.

Animals and Grouping

Male and female BALB/c mice, weighing 20–22 g, 3-4-week old, were purchased from the Shanghai SLAC Experimental Animals Inc of China (No. SCXK 2012-0002, specific pathogen-free grade). Animals were cared for in accordance with the guidelines of the National Science and Technology Committee of China. All procedures and animal experiments were approved by the Animal Care and Use Committee of the Animal Center of Zhejiang Chinese Medicine University (No. ZSLL-2010-66).

Totally 70 mice were divided into 7 groups with 10 mice in each group: normal, model, low-, medium-, high-dose PDS-C (20, 40, 80 mg/kg, L-, M-, H-PDS-C), cyclosporine (CsA, 40 mg/kg). The AA mouse model was established in all mice except those in the nomal group as previously described.[15] Briefly, BALB/c mice were exposed to 5.0 Gy total body irradiation at approximately 1.0 Gy/min, and injected via tail vein with a single dose of 2×10^6 lymphocytes isolated from lymph glands of DBA mice within 4 h after irradiation. On day 4 after establishment of AA model, PDS-C, CsA and andriol were intragastrically administered for 15 days at dosages abovementioned per day respectively, while the mice in the normal and model groups were administered with same volume of saline solution. The general condition of each group mice was observed such as activities, eating, fur, and so on after treatment.

Peripheral Blood Counts and Bone Marrow Pathological Examination

The blood samples were collected from the tail vein of mice, and peripheral blood of white blood cell (WBC), neutrophil (NEU) absolute value, platelets (PLT), red blood cell (RBC) counts and hemoglobulin (Hb) concentrations were measured by hematology cell counter (HORIBA ABX PENTRA XL 80, Montpellier, France) on day 0, 5, 10 and 15 after treatment. The mice were sacrificed by cervical dislocation after PDS-C treatment for 15 days, and the femurs were taken out. One femur of each mouse was used for histopathology evaluation. The femurs were fixed with picric acid for 24 h, decalcified with 10% hydrochloric acid for another 24 h, and dehydrated with 50%–95% alcohol, then processed by xylene, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). The bone marrow sections were examined by light microscopy.

Bone Marrow Semisolid Culture for Colony Forming Assay

The bone marrow nucleated cells were isolated from the femur and plated onto wells of tissue culture plates using a semisolid culture system for colony forming unit (CFU)-erythroid (CFU-E), CFU-granulocyte macrophage (CFU-GM) and CFU-megakaryocytic (CFU-MK). Briefly, the bone marrow cells were cultured in Iscove's modified Dulbecco's medium (IMDM) consisting of 20% fetal bovine serum, 300 mg/L glutamine, 10 μg/L recombinant murine GM colony stimulating factor or erythropoietin (GM-CSF or Epo, Peprotech, Israel), and 0.3% agar as viscous support. For CFU-MK culture,[13] the cells were cultured in IMDM medium consisting of 20% fetal bovine serum, 300 mg/L glutamine, 1% bovine serum albumin, 10^{-5} mol/L 2-mercaptoethanl, and 10 μg/L recombinant murine thrombopoietin (Tpo, Peprotech, Israel). The experiments were performed in triplicates with 10^5 nucleated cells per well, and incubated at 37 ℃ in a humidified atmosphere supplemented with 5% CO_2. The colony numbers of CFU-E (≥8 cells), CFU-GM (≥40 cells) were counted after 5-day culture. Murine megakaryocytes within CFU-MK colonies were

identified by acetylcholinesterase staining as previously described,[13] and the colony numbers of CFU-MK (≥4 cells) were counted after culture for 7 days.

Analysis of T Lymphocyte Subpopulation by Flow Cytometry

The blood samples of each mouse were collected from the tail vein after 15 days of treatment, and treated with 0.1% NH_4Cl to lyse the RBC. The cells were labeled with monoclonal antibodies against CD3-FITC, CD4-PE, CD8-PE-CY7 (eBiosience, USA); an isotype antibody was employed as a negative control. Mean fluorescence was measured using flow cytometry (BD FACS Calibur, USA). The cell suspensions from the spleens were obtained sterilely from the mice of each group. Briefly, the spleens were harvested, cut into small pieces, and grounded with a grinder. Removal of RBC and cell debris was accomplished by sequential filtration through 70 and 40 μm filters (BD Biosciences, USA), respectively.[15] Regulatory T cells (Treg) in the cell suspensions were assayed according to the manufacturer's instructions using a Treg assay kit (eBiosience, USA). Mean fluorescence was measured using flow cytometry.

Total Cell Lysate Preparation and Western Blot Analysis

After PDS-C treatment for 15 days, spleens of the mice in each group were harvested, cut into small pieces and ground with a grinder. Spleen cell lysate was prepared as previously described. Western blot was performed as previously described.[12-16] Briefly, 30 μg of protein samples were boiled and loaded into each lane, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. The proteins were transferred from SDS-PAGE gel to nitrocellulose membrane (Amersham, USA). After blocking, the membranes were incubated with primary antibodies against T-bet, GATA-3 and FOXP3 (Santa Cruz, USA), and then incubated with peroxidase conjugated secondary antibody (Santa Cruz). The specific bands were visualized by Luminol using ECL kit (Santa Cruz). The experiments were repeated 3 times.

Statistical Analysis

Data were analyzed using SPSS 17.0 (IBM, Armonk, NY, USA). All data were expressed as mean and standard deviation ($\bar{x} \pm s$). Student's *t*-test and one way ANOVA was used for statistical analysis. A value of $P<0.05$ was taken as statistically significant.

RESULTS

PDS-C Improved the General Condition of AA Mice

Mice in the normal group ate and behaved normally and had no weight loss. Mice in the model group were inactive, eating less, had dim eyes, weight and hair loss. However, after treated with medium- and high doses of PDS-C, the mice showed improvement on general condition than the model mice, revealing more active, ate more and had no weight loss.

PDS-C Increased WBC, NEU, PLT, RBC Counts and Hb Concentration of AA Mice

Mice in the model group showed severe pancytopenia with significant reduce on peripheral blood of WBC, NEU, RBC, PLT counts and Hb concentrations compared with the normal mice on day 5 to 15 (all $P<0.01$, Table 1). The WBC, NEU, PLT, RBC counts and Hb concentrations in the normal and model mice remain essentially unchanged. In contrast, WBC counts in 3 PDS-C treated groups increased, which were significantly higher than the model mice (all $P<0.01$). Among them, WBC counts in the M- and H-PDS-C groups were higher than the L-PDS-C group (all $P<0.01$). Similarly, for those mice in both CsA and andriol groups, WBC counts rose on day 5 to 15, as compared with the model mice ($P<0.05$ or $P<0.01$).

The peripheral blood NEU absolute value in 3 PDS-C treated groups increased on day 5 to 15, which were significantly higher than the model mice ($P<0.05$ or $P<0.01$). Among them, NEU absolute value in the M- and H-PDS-C groups were higher than the L-PDS-C group (all $P<0.01$). Similarly, for those mice in both CsA and andriol groups, NEU absolute value rose on day 10 and 15, as compared with the model group ($P<0.05$ or $P< 0.01$).

The peripheral blood PLT counts in 3 PDS-C treated groups increased on day 5 to 15, which were significantly higher than the model mice (all $P<0.01$). Among them, PLT counts in the M- and H-PDS-C groups were higher than the L-PDS-C group (all $P<0.01$). Similarly, for those mice in both CsA and andriol groups, PLT counts rose on day 5 to 15, as compared with the model mice ($P<0.05$ or $P<0.01$).

The peripheral blood RBC counts in the M- and H-PDS-C groups increased on day 5 to 15, which were

Table 1. PDS-C Increased WBC, NEU, PLT, RBC Counts and Hb Concentration in AA Mice after 5,10, 15 Days of Treatment ($\bar{x} \pm s$, n=10)

Group	Time	WBC (10^9/L)	NEU absolute value (10^9/L)	PLT (10^9/L)	RBC (10^{12}/L)	Hb (g/L)
Normal	Day 5	5.74±0.41	0.32±0.01	449.4±45.4	12.0±1.0	154.2±10.6
	Day 10	5.57±0.39	0.31±0.02	431.1±33	11.7±1.0	156.2±10.6
	Day 15	5.83±0.45	0.32±0.02	479.3±36.5	11.7±1.0	156.2±13.6
Model	Day 5	0.87±0.17*	0.02±0.01*	95.5±8.0*	7.8±0.8*	90.2±7.5*
	Day 10	0.81±0.13*	0.03±0.01*	89.6±6.48*	8.1±0.8*	91.2±8.5*
	Day 15	0.80±0.19*	0.03±0.01*	90.1±9.42*	8.2±0.9*	94.3±7.9*
L-PDS-C	Day 5	1.17±0.17△△	0.03±0.01△▲	164.8±24.3△△▲	8.1±0.7	100.4±8.3
	Day 10	1.41±0.16△△▲	0.04±0.01△	170.7±22.3△△▲	8.7±0.8	109.0±9.7
	Day 15	1.54±0.28△△▲	0.06±0.01△△▲	174.5±25.5△△	9.7±0.8	123.5±10.4△
M-PDS-C	Day 5	1.51±0.13△△▲	0.04±0.01△△▲	206.5±25.3△△▲	8.6±0.6	105.0±8.6△
	Day 10	1.73±0.83△△▲○	0.07±0.01△△▲	244.3±22.2△△▲	9.2±0.7	105.0±8.6△
	Day 15	2.93±0.16△△▲○	0.10±0.01△△▲○	256.6±20.5△△▲○	10.2±1.0△	134.6±11.5△△
H-PDS-C	Day 5	1.99±0.11△△▲○	0.05±0.01△△▲○	233.0±27.2△△▲○	8.8±0.7△	107.0±8.7△
	Day 10	2.65±0.35△△▲○	0.10±0.01△△▲○	293.5±29.4△△▲○	9.8±0.7△	120.0±8.7△△
	Day 15	3.21±0.17△△▲○	0.14±0.02△△▲○	314.0±31.3△△▲○	10.3±1.1△	147.4±10.7△△▲
CsA	Day 5	1.09±0.12△	0.02±0.01	117.2±23.5△	8.1±0.8	100.2±7.9
	Day 10	1.19±0.18△	0.04±0.01△	135.8±20.0△△	8.6±0.8	110.0±9.1△
	Day 15	1.28±0.18△△	0.04±0.01△	150.8±24.8△△	9.6±1.0	120.3±9.2△
Andriol	Day 5	1.31±0.12△△	0.04±0.01△△	178.8±18.1△△	8.6±0.8	110.0±8.6△
	Day 10	1.37±0.32△△	0.06±0.01△△	211.1±17.8△△	9.4±0.8	120.0±9.8△△
	Day 15	1.44±0.29△△	0.08±0.01△△	217.8±28.0△△	11.0±1.0△	142.6±12.5△△

Notes: *P<0.01, *vs*. normal mice; △P<0.05, △△P<0.01, *vs*. model mice; ▲P<0.01, *vs*. CsA group; ○P<0.01, *vs*. Andriol group

higher than the model mice (all P<0.05). There was no obvious change in CsA treatment group, whereas for andriol treated group, RBC counts increased on day 15, compared with the model mice (P<0.05).

The peripheral blood Hb concentration in the M- and H-PDS-C groups increased on day 10 and 15, which were significantly higher than model mice (P<0.05 or P<0.01). For those mice in the CsA group, Hb concentration increased on day 10 to 15, and andriol group increased on day 5 to 15, respectively, which were higher than model mice (P<0.05 or P<0.01).

PDS-C Reduced Myelosuppression in AA Mice

Figure 1 shows that hematopoietic cells on bone marrow histopathology after treatment. In normal mice, bone marrow sections appeared hematopoietic tissue hyperplasia, hematopoietic cells abundant

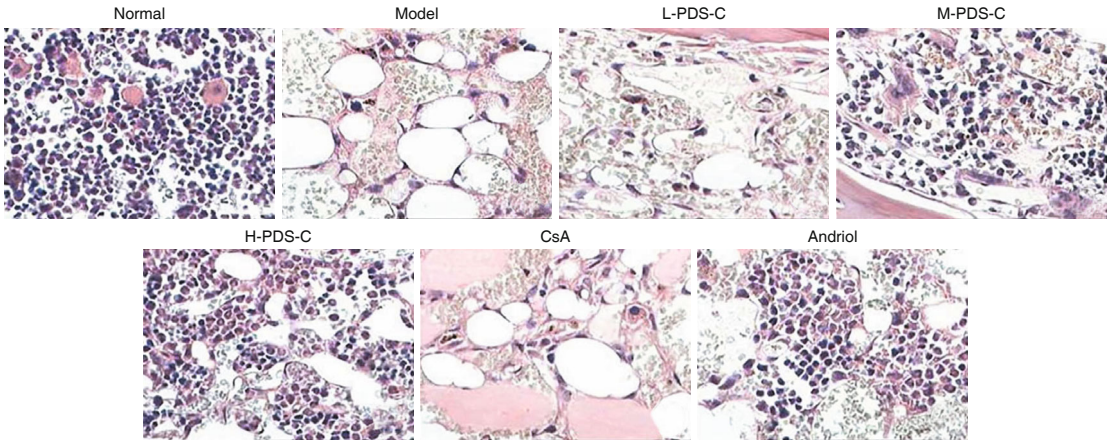

Figure 1. PDS-C Reduced Myelosuppression in AA Mice (HE staining, ×200)

with normal cellularity of the myeloid, erythroid and megakaryocytic lineages, and little or no discernable adipose cells. In contrast, in model mice, bone marrow biopsy sections showed low hyperplasia of decrease in cellularity with considerable replacement of hematopoietic tissue by adipose cells, only very few myeloid, erythroid and megakaryocyte cells are present. Treatment of AA mice particularly with 3 dosages PDS-C resulted in dose-dependently increase in bone marrow cellularity and hematopoietic cells of myeloid, erythroid and megakaryocytic lineages, and decrease in adipose cells. The increase in hematopoietic cells was only very modest with L-PDS-C, but was particularly prominent of hematopoietic cells in the M- and H-PDS-C groups compared with the model mice. Similar result with regard to the increase in hematopoietic cells was also observed in andriol-treated mice. However, the myelosuppression status of model mice was not obviously reduced in CsA-treatment group.

PDS-C Enhanced Proliferation of Hematopoietic Progenitor Cells in AA Mice

The bone marrow semisolid culture *in vitro* showed that CFU-E, CFU-GM and CFU-MK colony numbers were dramatically decreased in the model mice compared with the normal mice. Table 2 shows that colony formation of CFU-E, CFU-E and CFU-MK were enhanced in response to PDS-C treatment at low-, medium- and high doses, respectively, which were significantly higher than the model mice (all $P<0.01$). CsA treatment also increased colony formation of CFU-E, CFU-GM and CFU-MK, but was less than M- and H-PDS-C groups (all $P<0.01$). The effect of andriol was stronger in enhancing proliferation of erythroid progenitor cells, but weaker in promoting granulocytic and megakaryocytic progenitor cells compared with the H-PDS-C group (all $P<0.01$).

Table 2. PDS-C Increased Colony Numbers of CFU-E, CFU-GM and CFU-MK in AA Mice after 15 Days Treatment ($\bar{x}\pm s$, n=10)

Group	CFU-E (10^5 cells)	CFU-GM (10^5 cells)	CFU-MK (10^5 cells)
Normal	133.0±14.4	92.1±11.9	50.2±5.9
Model	21.8±2.5*	18.2±1.4*	10.1±2.1*
L-PDS-C	35.1±3.0△	30.0±2.8△	18.8±2.1△
M-PDS-C	42.6±2.4△▲	38.5±5.5△▲	24.8±3.6△▲○
H-PDS-C	62.5±3.5△▲○	53.0±5.0△▲○	38.0±5.6△▲○
CsA	34.5±3.5△	28.0±2.7△	17.1±2.8△
Andriol	46.0±2.8△	33.2±3.3△	20.2±4.2△

Notes: *$P<0.01$ *vs*. normal group; △$P<0.01$ *vs*. model group; ▲$P<0.01$ *vs*. CsA group; ○$P<0.01$ *vs*. andriol group

PDS-C Altered Percentage of T Cells Subsets in AA Mice

Table 3 and Figure 2 show the changes of T cell subsets in peripheral blood and spleen, following PDS-C treatment in AA mice. Compared with the normal mice, the percentages of $CD3^+$ and $CD3^+CD4^+$ cells of the peripheral blood were significantly decreased, and the percentages of $CD3^+CD8^+$ cells were significantly increased in the model mice (all $P<0.01$). However, $CD3^+$ and $CD3^+CD4^+$ cells were significantly increased in M- and H-PDS-C groups ($P<0.05$ or $P<0.01$), and $CD3^+CD8^+$ cells were decreased in response to PDS-C treatment at 3 dosages groups compared with the model mice (all $P<0.01$). The similar results were observed in mice with CsA treatment ($P<0.05$ or $P<0.01$).

$CD4^+CD25^+FOXP3^+$ cells (Tregs) in the spleen were significantly reduced in the model mice compared with the normal mice ($P<0.05$), and Tregs in M- and H-PDS-C groups reverted significant increase in the numbers towards near normal or normal levels, which were higher than model mice (all $P<0.05$). CsA treatment showed similar results compared with the

Table 3. PDS-C Altered Percentage of T Cells Subpopulation (%) of Peripheral Blood and Spleen in AA Mice ($\bar{x}\pm s$, n=10)

Group	$CD3^+$	$CD3^+$ $CD4^+$	$CD3^+$ $CD8^+$	$CD4^+$ $CD25^+$	Treg
Normal	48.1±4.1	98.2±9.4	5.4±2.4	3.9±0.4	86.2±8.4
Model	19.7±1.7**	76.3±7.4*	23.6±2.0**	14.1±1.0**	74.2±6.8*
L-PDS-C	32.0±3.1△△	82.2±7.6	9.0±0.8△△▲○	16.3±1.3▲	81.9±7.8
M-PDS-C	34.1±3.1△△▲○	86.1±8.2△	8.3±0.6△△▲○	13.3±1.1▲	84.4±7.9△
H-PDS-C	42.1±3.6△△▲○	95.6±8.7△△○	5.1±0.4△△▲○	10.3±0.9△▲○	88.7±8.6△
CsA	27.4±2.4△	95.7±8.9△△	3.6±0.4△△	6.1±0.4△△	85.1±7.9△
Andriol	27.1±2.6△	82.4±8.1	14.6±1.2△	13.8±1.0	80.2±7.5

Notes: *$P<0.05$, **$P<0.01$ *vs*. normal group; △$P<0.05$, △△$P<0.01$, *vs*. model group; ▲$P<0.01$, *vs*. CsA group; ○$P<0.01$ *vs*. andriol group

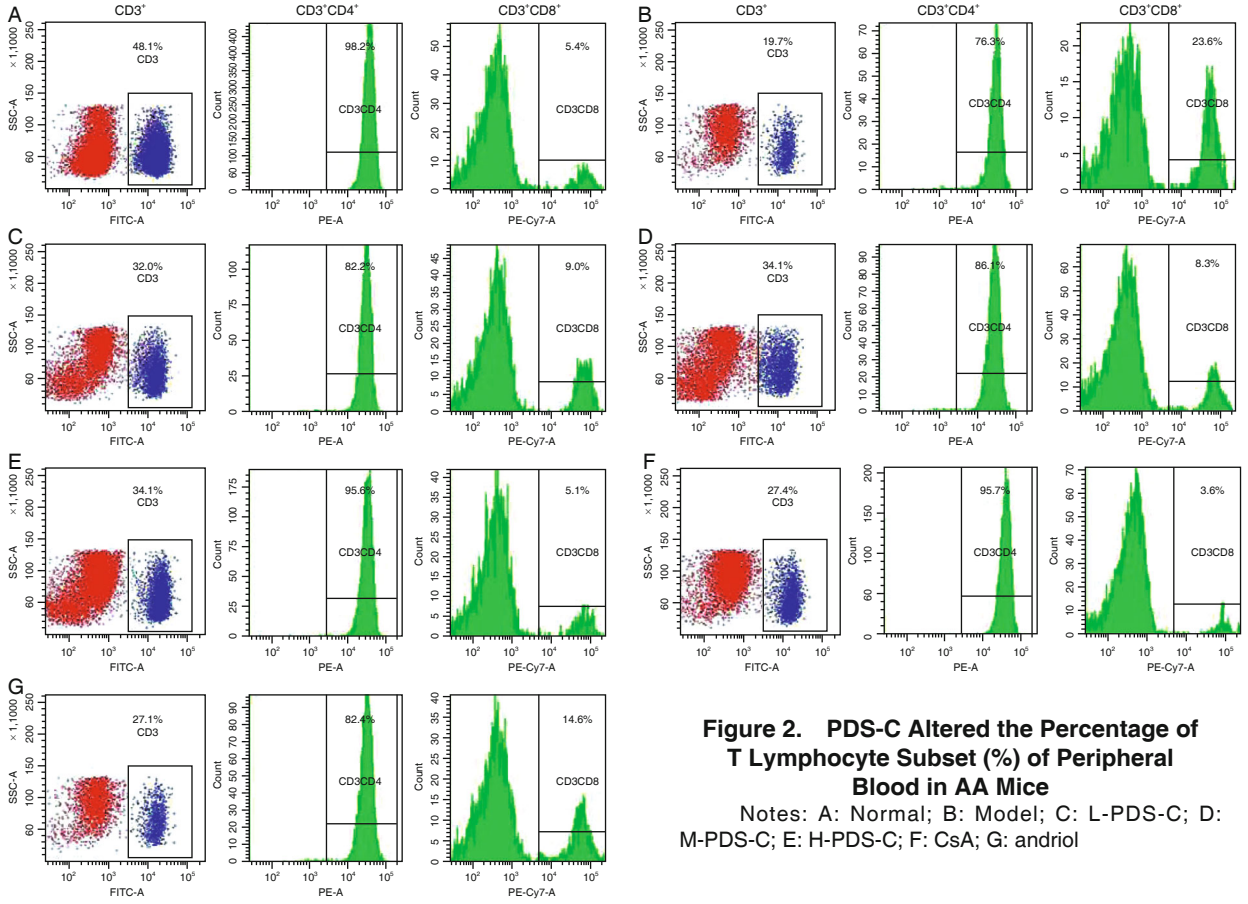

Figure 2. PDS-C Altered the Percentage of T Lymphocyte Subset (%) of Peripheral Blood in AA Mice

Notes: A: Normal; B: Model; C: L-PDS-C; D: M-PDS-C; E: H-PDS-C; F: CsA; G: andriol

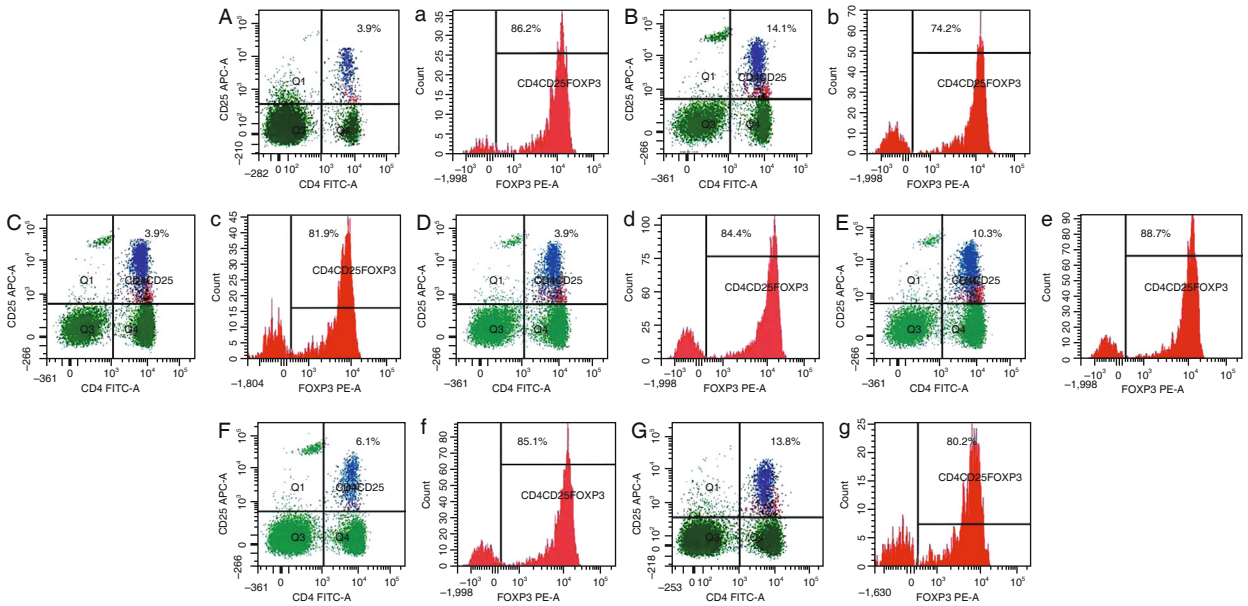

Figure 3. PDS-C Increased the Percentage of Spleen $CD4^+CD25^+FOXP3^+$ Cells in AA Mice

Notes: A,a: Normal; B,b: Model; C,c: L-PDS-C; D,d: M-PDS-C; E,e: H-PDS-C; F,f: CsA; G,g: andriol

model mice (P<0.05, Table 3 and Figure 3).

PDS-C Regulated T-bet, GATA-3 and FOXP3 Proteins Expression in AA Mice

Western blot analysis showed T-bet, GATA-3 and FOXP3 proteins expression levels in spleen cells from PDS-C-treated mice (Figure 4). T-bet protein was downregulated in spleen cells in PDS-C at medium- and high dosage groups compared with the model group (P<0.01). Similar results were obtained by CsA

and andriol treatment. In contrast, expressions of GATA-3 and FOXP3 proteins were upregulated in 3 PDS-C treated groups compared with the model group (P<0.05 or P<0.01).

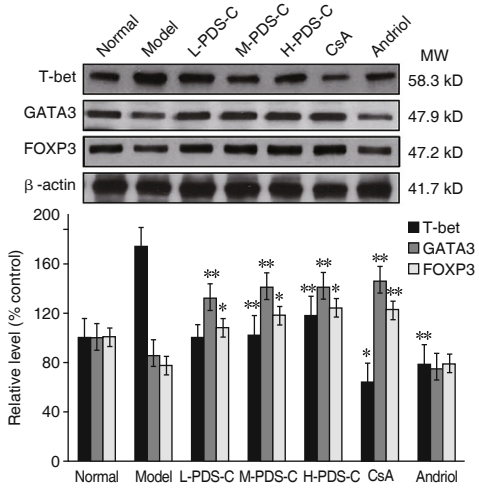

Figure 4. PDS-C Regulated Expression of T-bet, GATA-3 and FOXP3 Proteins in AA Mice

Notes: $^{*}P$<0.05, $^{**}P$<0.01, *vs*. model group

DISCUSSION

AA is an immune-mediated disease due to a breakdown of self-tolerance leading to an expansion of oligoclonal cytotoxic T cells which target hematopoietic stem and progenitor cells, leading to their death by apoptosis and resulting in bone marrow failure.[17,18] In some situations, compromised stromal cell function due bystander effects also contributes to the bone marrow failure. As a consequence of bone marrow failure, patients with AA have severe anemia, increased risk of bleeding and infection.[19]

T cells play a crucial role in the immune destruction of bone marrow tissue in AA patients, peripheral blood $CD4^+$ T cells are often decreased, while $CD8^+$ cells are usually increased.[17,18] These cells secrete Th1 cytokines such as interferon-γ (IFN-γ), IL-2 and TNF-α, which inhibit proliferation of bone marrow hematopoietic stem and progenitor cells, inducing their apoptosis and causing bone marrow failure. Up-regulation of T-bet, a transcription factor that binds to the promoter region of IFN-γ gene, further contributes to the pathogenesis of AA. Bu regulating and suppressing auto-reactive T cells, Treg often decreased in number and function in AA, resulting in immune dysregulation, expansion of oligoclonal cytotoxic T cells and consequent destruction of bone marrow hematopoietic tissue and pancytopenia.[20,21]

We have reported that PDS-C was effective on promoting proliferation of the 3 lineages of hematopoietic progenitor cells, increasing colony formation of erythroid, granulocytic and megakaryocytic progenitor cells on bone marrow culture after PDS-C therapy.[11-14] Furthermore, our previous studies showed that PDS-C was effective in promoting proliferation and differentiation of human $CD34^+$ hematopoietic stem/progenitor cells, implying that PDS-C possess growth factor-like activity and synergistic effect with hematopoietic growth factors.[11,12,22]

In this study, we used a mouse model of immune mediated AA to investigate the effects of PDS-C on hematopoiesis. The results showed that PDS-C effectively and dose-dependently increased the peripheral blood WBC, PLT counts and NEU absolute value, as well as raised the colony numbers of CFU-GM, CFU-E and CFU-MK following bone marrow culture of hematopoietc progenitor cells. Histopathological examination of bone marrow revealed that PDS-C alleviated myelosuppression of bone marrow and accelerated its recovery in mice treated with moderate and high doses groups. In PDS-C treated mice, the bone histopathological sections showed increased cellularity and near normal hematopoietic activities of the myeloid, erythroid and megakaryocytic lineages, as well as significant reduction in adipose cells when compared with AA model mice. Our results suggested that PDS-C at moderate and high doses are at least as effective as andriol in promoting bone marrow hematopoietic recovery. Regarding the mechanism of androgen therapy in bone marrow failure, Rodrigo, et al[23] reported that androgen acts on the TERT gene and increases its telomerase activity.

We also measured changes of T cell subsets in the peripheral blood and spleen after PDS-C treatment. Our results indicated that PDS-C reduced peripheral blood $CD8^+$ cells, and increased $CD4^+$ cells, therefore, reverting the ratio of $CD4^+/CD8^+$ cells almost close to normal level. PDS-C treatment increased the percentage of $CD3^+$ $CD4^+$ cells and reduced $CD3^+CD8^+$ cells. PDS-C at moderate- and high doses raised significantly $CD4^+CD25^+FoxP3^+$ cells (Tregs). In particular, high-dose PDS-C treatment changed T lymphocyte subsets levels close to normal. Our data indicate that PDS-C may be as effective as immunosuppressant CsA in regulating T lymphocyte subpopulation.

The expression levels of T-bet, GATA-3 and PoxP3 proteins of spleen cells were analyzed by Western blot. T-bet is a type Th1 lymphocyte-specific transcription factor, which promotes the growth of Th1 cells through activating Th1 genetic program.[24] Solomou, et al[25] reported upregulated expression of T-bet in AA patients. Kaito, et al[26] found that downregulated expression of T-bet in AA patients had response to immunotherapy. Our results showed that T-bet protein level in spleen cells of AA model mice was abnormally high, while T-bet protein levels in both PDS-C and CsA-treated mice were lower compared with AA model mice.

GATA-3 is a transcription factor specific to Th2 cells and positively regulates Th2 development.[27] GATA-3 expression levels of spleen cells in PDS-C at high-, medium dose and CsA groups were clearly increased compared with model mice, suggesting that PDS-C could upregulate the GATA-3 transcription factor to stimulate differentiation of Th2 cells. In this regard, the effect of PDS-C was similar to CsA treatment. Treg plays a key role in immune regulation and tolerance.[28-30] Peripheral blood Treg in AA patients are decreased, and can be increased in response to immunotherapy. FoxP3 is a specific transcription factor in Treg, and Treg number and function are decreased in FoxP3 knockout mice. Our results showed expression of FoxP3 protein upregulated in PDS-C treated mice with moderate- and high doses groups compared with model mice, suggesting that PDS-C may correct the immune dysregulation by upregulation of FoxP3 expression, increasing Treg and improving bone marrow hematopoietic function.

As an immunosuppressing agent, CsA is currently used in treatment of patients with AA. It is effective in correcting the immune dysregulation in AA, and promoting recovery of bone marrow hematopoietic function.[29] In this study, CsA was used as positive control, the results of PDS-C-treated AA mice appeared to be as effective as CsA treatment, suggesting that PDS-C may possess CsA-like activity in regulating the immune functions. We hypothesized that PDS-C may correct T cell subset imbalance by downregulating T-bet and upregulating GATA-3 and FoxP3 expression, consequently promoting the recovery of hematopoietic function. In this study, bone marrow hematopoietic activities in CsA-treated mice were inferior to both PDS-C and andriol-treated mice, respectively. The possible explanation is that CsA may be effective in correcting the immune dysregulation, but not as effective in enhancing hematopoiesis, since our myelosuppression of AA model was caused by both radiation and infusion lymphocytes of DBA mice. These findings were consistent with the use of effective combination CsA and androgen in treating patients with AA in China.

In conclusion, our results indicate that PDS-C promotes recovery of the suppressed hematopoiesis, reverses T cell subset imbalance, and increases Treg in AA mice. The current data together with our previous experimental findings, demonstrate that PDS-C possesses dual activities: promoting proliferation hematopoietic progenitor cells (similar to androgen and hematopoietic growth factors), and modulating T lymphocyte dysfunctions (similar to CsA).

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Gao RL, Zheng ZY and Chong BH conceived of the study, designed, supervised the experiments and wrote the manuscript. Yu XL performed preparation and analysis of PDS-C. Dai TY, Yin LM, Zhao YN, Xu M and Zhuang HF performed animal and cell experiments.

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(Accepted December 11, 2018)
Edited by TIAN Lin