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Original Article

Establishment of a new mouse model of systemic lupus erythematosus complicated by atherosclerosis

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Abstract: Background: Atherosclerosis (AS) is a common complication of systemic lupus erythematosus (SLE). However, its pathogenic mechanism is not clear. Related clinical and basic research is limited by the lack of a reliable mouse model. We aimed to establish a reliable and stable mouse model of SLE complicated by AS, similar to that seen in clinical practice. Methods: Ten-week-old LDLr$^{-/-}$ female mice were divided into two groups. The model group was injected intraperitoneally with 0.5 mL of Pristane reagent and the control group with 0.5 mL saline. Both groups began a high-fat diet at the same time and continued it for 14 weeks after injection. The weights of the two groups of mice were recorded before injection and before death. Serum lipids, antinuclear antibody (ANA), anti-double-stranded DNA (dsDNA) antibody, and interleukin (IL)-17, IL-23, IL-12, and IL-10 levels were detected, and histopathological changes in the kidney, spleen, and carotid artery were observed. The rank sum test between groups was used for statistical analysis, and the analysis of variance was repeated with multiple measurements within each group. Differences with $P<0.05$ were considered statistically significant. SPSS software (IBM, Armonk, NY, USA) was used for statistical analysis and GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA) for illustrating the figures. Results: During the modeling process, hair-shedding was observed in the model mice. After modeling, mice activity gradually decreased and they presented positive ANA and anti-dsDNA antibodies and white lumps on the surface of their abdominal organs. The splenomegaly was obvious, the lymph nodes were hyperplastic, the medullary sinus was irregular, and there was inflammatory cell infiltration in the spleen. The average values of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and immunoglobulin G in the model group were higher than those in the saline group. Renal pathology revealed inflammation with glomerular epithelial hyperplasia, scattered interstitial lymphocyte infiltration, and fibrous tissue hyperplasia. Carotid artery histopathology showed thickening of the intima, adhesion of arterial plaque, and cholesterol crystals and calcification in the plaque. The proteinuria proportion was higher in the model group. In the normal saline group, the blood lipid levels were high, but ANA and anti-dsDNA antibodies were negative and the mice had no hair loss and presented normal activity. Conclusions: Injection of LDLr$^{-/-}$ mice with Pristane successfully induced a model of SLE complicated by AS, which may allow basic research of this condition in the future.

Keywords: Systemic lupus erythematosus, atherosclerosis, mouse model, antinuclear antibodies, anti-double-stranded DNA antibodies, interleukins, T helper 17 cells, regulatory T cells

Introduction

Atherosclerosis (AS) is a common complication in many patients with systemic lupus erythematosus (SLE). Its incidence has been increasing and attracting the attention of many scholars. Some studies have shown that the incidence of cardiovascular diseases (CVD) in patients with SLE is higher and one of the long-term causes of death [1]. A clear cause for SLE has not been found so far, but patients with SLE and AS are not affected by only ordinary hyperlipidemia and hypertension. Although the survival time of patients with SLE has been significantly prolonged, many long-term and chronic complications, especially cardiovascular and cerebrovascular diseases caused by AS, significantly affect the prognosis of patients [2-4]. Our previous studies found that the prevalence of AS in SLE patients was as high as 12%, and the median age of onset was 41.09±18.53 years. This also confirmed that SLE patients not only had a

high incidence of AS but also had an early onset trend [5]. Therefore, SLE complicated by AS has become a hot topic in this field. However, its pathogenic mechanism is not clear, even though studies have shown that the imbalance between T helper 17 (Th17) cells and regulatory T (Treg) cells plays an important role in the early onset of AS in SLE [6, 7]. In addition, cytokines such as interleukin (IL)-23, IL-12, and IL-10 play a certain role in promoting the pathogenic process and affecting the immune balance of the body [8]. Currently, most of the research on the disease is clinical and basic research is limited due to the inability to create a stable and reliable mouse model. Therefore, this study was dedicated to establishing a long-term, stable, and reliable mouse model similar to clinical SLE complicated by AS. This was confirmed by observing the clinical manifestations of the experimental process in mice and detecting the physiological and pathological indicators of the onset of SLE and AS in the mice after modeling.

Materials and methods

The study was approved by the Ethics Committee of the Guilin.

Experimental animals

Twenty, 8-week-old SPF grade LDLr$^{-/-}$ mice were purchased from Jiangsu Jicui Yaokang Biotechnology Co., Ltd (Jiangsu province, China) and raised on ordinary feed until the 10th week of age.

Reagents

Pristane reagent was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Antinuclear antibody (ANA), anti-double-stranded DNA antibody (anti-dsDNA), IL-17A, IL-23, IL-10, and IL-12 kits were purchased from Wuhan Huamei Bioengineering Co., Ltd. (Hubei province, China) under the CUSABIO brand. Immunohistochemical antibodies were purchased from Origene (Rockville, MD, USA). Urine protein test paper was purchased from Unislite Co., Ltd. (China). High-fat feed was purchased from Beijing Keao Xieli Feed Co., Ltd. (Beijing province, China). Mouse peripheral blood lymphocyte separation solution was purchased from Beijing Solarbio Technology Co., Ltd. (Beijing province, China). Flow cytometric intranuclear and intracellular kits, flow cytometry antibody BB700 CD4, BV421 IL-17A, PE Foxp3, control antibodies, PMA, BFA, and blocker Fc were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA).

Animal grouping and establishment of SLE complicated by AS model

During the whole experiment, all mice were kept in the Experimental Animal Center of Guilin Medical University at SPF grade and with an ear tag mark. The 8-week-old LDLr$^{-/-}$ mice were fed until they were 10 weeks of age and were randomly divided into two groups. The 10 LDLr$^{-/-}$ mice in the model group were injected intraperitoneally once with 0.5 mL Pristane solution and the other 10 mice in the control group were injected intraperitoneally once with 0.5 mL normal saline. On the same day, a high-fat diet was started and continued for 14 weeks.

Animal model generation and indicator detection after 14 weeks of modeling

Weight measurement of mice: The body weights of the two groups of mice were measured and recorded before injection and at the end of 14 weeks of modeling, and the difference in body weight of each group before and after was calculated for statistical analysis.

Measurement of proteinuria in mice: Proteinuria was measured with protein test paper every four weeks before and after Pristane and normal saline injection. According to the chromogenic degree of the reaction area, urine protein was classified as negative (-), 150 mg/L (±), 300 mg/L (+), 1000 mg/L (++), 3000 mg/L (+++), or ≥5000 mg/L (++++).

Detection of serum lipids (TC, TG, HDL-C, and LDL-C), IgG, and complement C3 in mice: After intraperitoneal injection, the mice were fed a high-fat diet for 14 weeks, weighing approximately 30 g. They were then anesthetized by intraperitoneal injection of 400 mg/kg 4% chloral hydrate and were euthanized by removing the eyeballs and bleeding, and collecting blood for experiments. The blood volume was about 1 ml. After removing the carotid artery, spleen, and kidney of the mouse, the carcass was stored in a designated refrigerator in the

laboratory. The blood was precipitated for at least 30 min and then centrifuged to collect the serum. Serum samples were collected and sent to the Laboratory Department of the Affiliated Hospital of Guilin Medical College for the measurement of serum lipids (total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), immunoglobulin G (IgG), and complement C3.

Measurement of mice ANA and anti-dsDNA antibody, IL-17, IL-23, IL-12, and IL-10 serum levels by ELISA: After modeling the mice, blood was collected by tail-cutting after anesthesia with 10 ml/kg of 4% chloral hydrate at 4, 8 and 12 weeks, about 450 ul, and the serum was stored at -80°C after centrifugation. The test was carried out according to kit instructions: ① All reagents and samples were brought to room temperature before use. The sample was centrifuged again after thawing and before the assay. All samples and standards were assayed in duplicate. ② All reagents, working standards, and samples were prepared as directed in the previous sections. ③ The Assay Layout Sheet was verified to determine the number of wells to be used and any remaining wells and the desiccant were placed back into the pouch and this was sealed with the ziplock and stored at 4°C. ④ A volume of 100 µL of the standard and sample was added per well and these were covered with the adhesive strip provided. The cells were incubated for 2 h at 37°C. The standards and samples assayed were recorded in the provided plate layout. ⑤ The liquid was removed from each well without washing. ⑥ Then 100 µL of biotin-antibody (1×) was added to each well and these were covered with a new adhesive strip. The cells were incubated for 1 hour at 37°C. The mixture was warmed to room temperature and mixed gently until the solution appeared uniform. ⑦ Each well was aspirated and washed by filling each well with the Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and letting it stand for 2 min; the process was repeated two times for a total of three washes. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting, and the plate was inverted and blotted against clean paper towels. ⑧ Then 100 µL of horseradish peroxidase-conjugated avidin (1×) to each well. The microtiter plate was covered with a new adhesive strip and the cells were incubated for 1 h at 37°C. ⑨ The aspiration/wash process was repeated five times as in step ⑦. ⑩ Next, 90 µL of TMB substrate was added to each well and incubated for 15-30 minutes at 37°C protected from light. ⑪ Finally, 50 µL of Stop Solution was added to each well and the plate was gently tapped to ensure thorough mixing. ⑫ The optical density of each well was determined within 5 min using a microplate reader set to 450 nm.

Histopathological changes of kidney, spleen, and carotid artery in mice: The kidneys, spleens, and carotid arteries of mice in each group were fixed in 4% tissue fixative for 72 h, dehydrated in alcohol, embedded in paraffin, and sectioned into slices with a thickness of 5 µm. The carotid artery and spleen were stained with hematoxylin-eosin (HE) and the kidney with HE, periodic acid-Schiff (PAS), and Masson. Histopathological changes were observed under a light microscope.

Immunohistochemical detection of the carotid artery in mice: Experimental steps: ① Dewaxing; ② Antigen repair; ③ The slice rack was took out, placed in 3% H_2O_2 solution, and inactivated in the incubation box for 10 min at room temperature; ④ The slice rack was rinsed 5 times with 1× Tris-buffered saline (TBS), span dry, wiped clean, 50 µL of 5% serum was added to it, and it was humidified at room temperature during 20 min for blocking; ⑤ The excess liquid was absorbed using absorbent paper (taking care not to dry the film), 50 µL of diluted primary antibody was added, and the rack was sealed in a humidified box at room temperature for 1 h; ⑥ The rack was rinsed five times with 1× TBST, 50 µL of secondary antibody was added, and the first was incubated in a humidified box at room temperature for 30 min; ⑦ The slice rack was rinsed five times with 1× TBS , dripped with 3,3' diaminobenzidine color developing solution, and rinsed quickly after 3 min; ⑧ The rack was dripped with one drop of Mayor's hematoxylin, counterstained for 1 min, and washed with TBS solution or tap water for 5 min; ⑨ Alcohol gradient and xylene dehydration: the slice rack was successively soaked in 60%, 80%, 95%, and 100% alcohol for 5 min, then soaked in xylene (III) for 5 min, and xylene (IV) for more 5 min. The film was sealed with neutral gum and observed under a microscope.

Detection of the number of $CD4^+CD25^+$Treg and $CD4^+IL17^+$Th17 cells in peripheral blood of $LDLr^{-/-}$+Pristane mice by flow cytometry: Intracellular detection: ① Peripheral blood lymphocyte separation solution was used to extract mouse peripheral blood mononuclear cells, and one tube of naked cells (10^5 cells/tube) was set aside; ② 1 μL of PMA, BFA, and Fc blocker was added per 1 mL of cell culture medium and mixed thoroughly to stimulate the cells, and cultures were evenly incubated at 4°C for 15 min; ③ Cells were washed, 5 μL of CD4 specific fluorescent antibody for surface staining was added, and the cultures were incubated at 4°C for 30 min; ④ Cells were resuspended, 100 μL/well of Fixation/Permeabilization solution was added, and the cultures were incubated at 4°C for 20 min; ⑤ Cells were washed twice with 1× BD Perm/Wash™ buffer 250 μL/time; ⑥ The fluorescent antibody group or negative control group plus 5 μL of the corresponding antibody were set up, diluted to 50 μl with BD Perm/Wash™ Buffer, and incubated at 4°C for 30 min protected from light; ⑦ 1× BD Perm/Wash™ Buffer 250 μL/time was used to wash the cells twice, then these were resuspended in 250 μL of Staining Buffer, and detected using the equipment.

Intranuclear detection: ① The single-cell suspension (10^6 cells) was resuspended with 100 μL of BD Pharmingen™ Stain Buffer, and one tube of naked cells (10^5/tube) was set aside; ② Five μL of cell surface fluorescent antibody CD4 was added per tube and incubated for 30 min in the dark at 4°C. Then, 2 mL of stain buffer was added to wash the cells; ③ One mL of freshly prepared 1× Fix/Perm Buffer working solution was added, shaken and mixed for 3 s, and incubated at 2-8°C in the dark for 40-50 min to fix the membrane; ④ One mL of 1× Perm/Wash™ Buffer was added three times, the suspension was centrifuged at 350 *g* for 6 min at 2-8°C, and the supernatant was discarded; ⑤ Next, 100 μL 1× Perm/Wash™ Buffer was used to resuspend the cells, 5 μL of the specific intracellular factor antibody Foxp3 or nonspecific control was added to the tube, this was vortexed for 10 sec for appropriate mixing, and incubated at 2-8°C in the dark for 40-50 min. ⑥ 2 mL of 1× Perm/Wash™ Buffer was added and the tube was washed twice, 350 g, centrifuged at 2-8°C for 6 min, and the supernatant was discarded. ⑦ A total of 350 μL of stain buffer was used to resuspend the cells and these were detected using the equipment.

Statistical analysis

The rank sum test between groups was used for statistical analysis, and the analysis of variance was repeated with multiple measurements within each groups. Differences with $P<0.05$ were considered statistically significant. SPSS software (IBM, Armonk, NY, USA) was used for statistical analysis and GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA) for illustrating the figures.

Results

Clinical manifestations of mice in the modeling process

Four weeks after the intraperitoneal injection, the modeled mice began to lose hair and their mobility began to slow down, including a decrease in cage climbing. Most of them lost their back hair and a few of them their cheek, head-top, and abdomen hair. In the 14th week of modeling, the anatomic analysis showed that a large number of white calcifications were attached to the abdominal cavity and thoracic organs of mice. The calcifications in the model group were more extensive than those in the control group, and some mice had pleural effusion. In addition, the model group presented severe splenomegaly, as shown in **Figure 1**.

Changes in the weight of mice

The body weights of mice in the two groups were measured before modeling and at the 14th week of modeling, and the weight difference between the two groups before and after modeling was calculated, as shown in **Table 1**. Rank sum test analysis showed that the weight difference between the two groups before and after modeling was statistically significant (**Figure 2**).

Proteinuria in mice

Urine protein was measured before the intraperitoneal injection and every four weeks after injection, and the rank sum test between the two groups had a $P<0.05$ value, suggesting a statistically significant difference (**Table 2**). The frequency of proteinuria in mice each week after the intraperitoneal injection of Pristine was significantly different from that before the injection, while the control group showed no statistically significant difference. **Figure 3** shows a scatter plot of proteinuria frequency in mice.

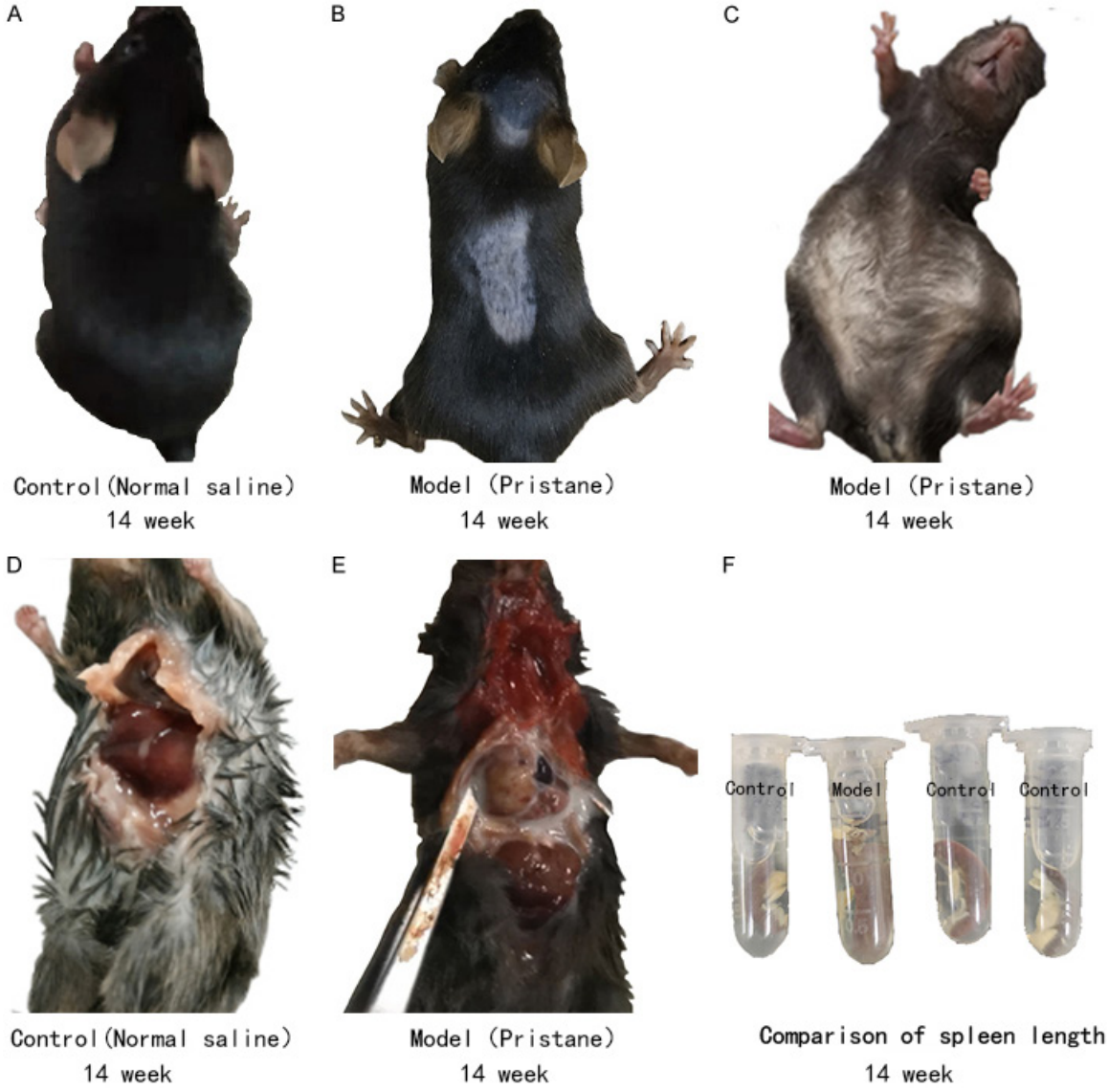


Figure 1. Clinical manifestations in mice of the model and control groups.

Table 1. Weight differences before and after modeling

Group	Weight difference										Mean ± standard deviation
Model (Pristane)	8.5	9.6	10	4.8	7.1	6.0	7.7	8.4	5.7	5.2	7.3±1.7
Control (normal saline)	6.2	3.7	3.4	3.4	3.9	5.7	3.5	3.9	3.9	3.3	4.1±1.0

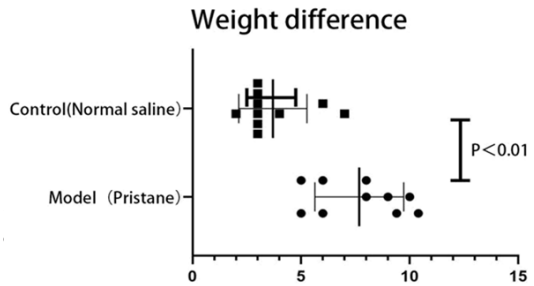


Figure 2. Weight differences between the two groups before and after modeling. As shown in the figure, the weight difference distribution in the model group was greater than that in the control group. The weight difference between the two groups was statistically significant (P<0.01).

Blood lipid (TC, TG, HDL-C, and LDL-C), IgG, and complement C3 levels in mice are shown in

Table 2. Follow-up test results of proteinuria in mice

Detection time	Group	Number of occurrences					
		-	±	+	++	+++	++++
Before modeling	Model (Pristane)	10	0	0	0	0	0
	Control (normal saline)	10	0	0	0	0	0
Modeling 4 weeks	Model (Pristane)	0	3	4	1	1	1
	Control (normal saline)	9	1	0	0	0	0
Modeling 8 weeks	Model (Pristane)	0	2	2	1	2	3
	Control (normal saline)	8	2	0	0	0	0
Modeling 12 weeks	Model (Pristane)	0	0	2	3	2	3
	Control (normal saline)	6	3	1	0	0	0
Modeling 14 weeks	Model (Pristane)	0	0	1	4	1	4
	Control (normal saline)	6	2	2	0	0	0

According to the chromogenic degree of the reaction area, urine protein was classified as negative (-), 150 mg/L (±), 300 mg/L (+), 1000 mg/L (++), 3000 mg/L (+++), or ≥5000 mg/L (++++).

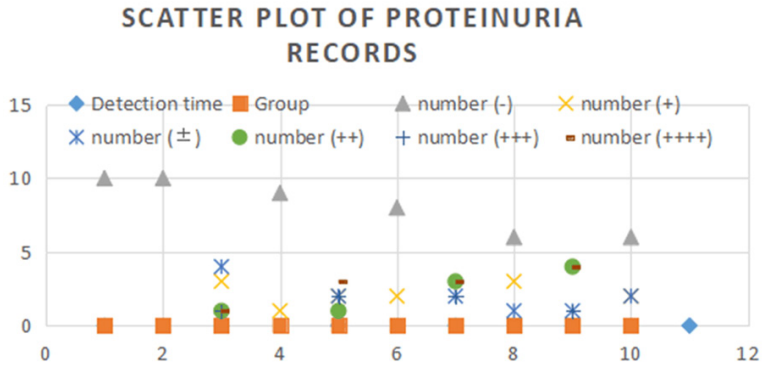


Figure 3. Proteinuria records.

Table 3. Serum lipid, immunoglobulin (Ig)G, and complement C3 levels

Factor	Model (Pristane)	Control (normal saline)
TG (mmol/l)	3.66±0.94	1.67±0.92*
TC (mmol/l)	20.71±3.89	16.77±2.39*
HDL-C (mmol/l)	3.32±0.89	3.63±0.84
LDL-C (mmol/l)	7.25±1.48	5.10±1.81*
IgG (g/l)	0.33±0.13	0.24±0.06
C3 (g/l)	0.14±0.04	0.17±0.07

Note: *means P<0.05 in the comparison between the two groups. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Table 3. The rank sum test suggested that the differences in HDL, IgG, and C3 levels in the different groups were not statistically significant (P+654>0.05), while the differences in TC, TG, and LDL-C were statistically significant (P< 0.05). **Figure 4B** and **4A** indicate that the average values of TC, TG, LDL, and IgG in the model group were higher than those in the control group, and the average values of HDL-C and C3 were lower than those in the control group.

Detection of IL-17, IL-23, IL-12, IL-10, ANA, and anti-ds-DNA antibodies in mice

The OD values of serum IL-17, IL-23, IL-12, IL-10, and ANA and anti-dsDNA antibodies in mice were determined using an ELISA kit and the concentrations were calculated using a standard curve. The rank sum test between the groups indicated that the differences between the two groups in the inflammatory factors measured every 4 weeks were statistically significant (**Table 4**). Repeated measurement analysis of variance showed that the expression of each inflammatory factor significantly increased with age after the intraperitoneal injection of Pristine and was significantly higher than that before the injection (**Figure 5**).

HE, Masson, and PAS staining of mouse kidneys

The histopathological examination of mouse kidneys by HE, Masson, and PAS staining showed that some glomeruli were enlarged, the number of cells increased, the basement membrane thickened, and the boundary with the surroundings unclear. The renal tubular epithelial cells had proliferated and were arranged in a disorderly manner. The cavity was narrow and irregular. The renal interstitium showed edema with inflammatory cell infiltration, but there was no significant difference in fibrosis between the two groups (**Figure 6**).

HE staining of mouse spleens

Histological HE staining of mouse spleens showed that the model mice had enlarged splenic white pulp area, enlarged lymph nodes,

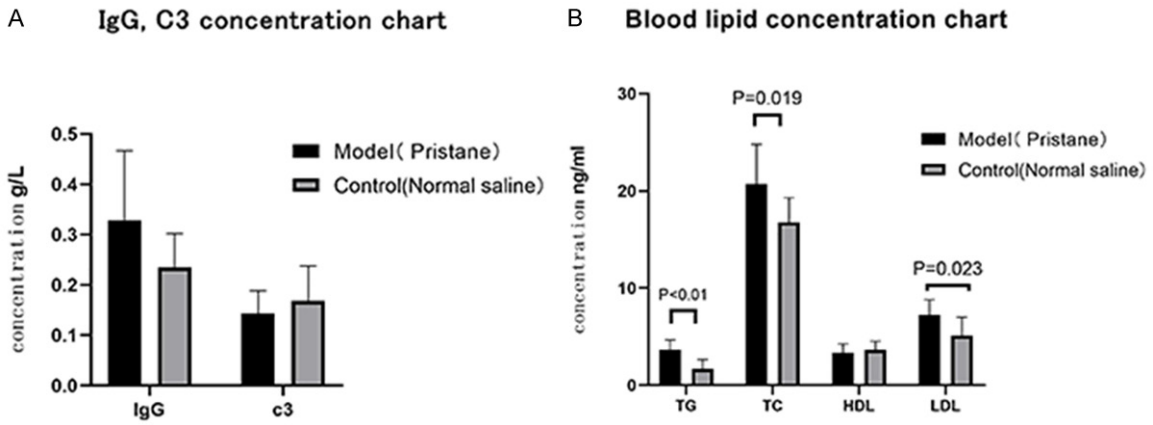


Figure 4. A. IgG and C3 concentration. B. Blood lipid concentration.

Table 4. Expression of inflammatory factors after intraperitoneal injection

Time	Factor	Model (Pristane)	Control (normal saline)
week 4 after intraperitoneal injection	IL-17 (pg/ml)	84550.00±2690.45	65800.00±5238.51*
	IL-23 (pg/ml)	5025.45±725.43	3718.18±331.39*
	IL-10 (pg/ml)	978.93±77.01	742.62±77.04*
	IL-12 (pg/ml)	7971.92±1240.96	6443.08±523.95*
	Anti-dsDNA antibody (pg/ml)	8120.50±558.37	1617.78±540.24*
	ANA (pg/ml)	19786.55±2513.21	11806.72±1666.78*
week 8 after intraperitoneal injection	IL-17 (pg/ml)	92330.00±2078.00	71740.00±4320.23*
	IL-23 (pg/ml)	7439.09±1254.57	4572.73±382.32*
	IL-10 (pg/ml)	1292.98±163.80	850.12±83.21*
	IL-12 (pg/ml)	11596.54±1618.68	6668.46±529.68*
	Anti-dsDNA antibody (pg/ml)	10260.63±736.71	1638.50±540.51*
	ANA (pg/ml)	27310.92±2955.25	11774.79±2396.80*
week 12 after intraperitoneal injection	IL-17 (pg/ml)	94530.00±1632.21	70800.00±3414.38*
	IL-23 (pg/ml)	7581.82±1209.71	5758.18±509.35*
	IL-10 (pg/ml)	1334.64±149.29	871.43±81.25*
	IL-12 (pg/ml)	11878.46±1473.83	6942.31±752.41*
	Anti-dsDNA antibody (pg/ml)	10422.57±741.65	1639.59±532.04*
	ANA (pg/ml)	27903.36±2791.21	11935.29±1831.41*
week 14 after intraperitoneal injection	IL-17 (pg/ml)	94500.00±2118.96	70690.00±5327.37*
	IL-23 (pg/ml)	7490.00±1307.48	5872.73±465.40*
	IL-10 (pg/ml)	1341.67±159.14	883.10±103.23*
	IL-12 (pg/ml)	11841.15±1520.22	6863.08±621.42*
	Anti-dsDNA antibody (pg/ml)	10696.84±699.91	1657.58±543.74*
	ANA (pg/ml)	28297.48±2711.97	12020.17±1604.33*

Note: * means P<0.01 in the comparison between the two groups. ANA, anti-nuclear antibody; anti-dsDNA, anti-double-stranded DNA antibody; IL, interleukin.

increased infiltration of lymphocytes and inflammatory cells, and more irregular sinuses (**Figure 7**).

HE staining of mouse carotid arteries

HE staining showed that both the model and control groups had a thickened carotid artery intima with lipid deposits, cholesterol crystals, and calcification deposits, but these features were more pronounced in the model group than in the control group (**Figure 8**). The immunohistochemistry results indicated that IL17 expression in the carotid atherosclerotic plaque was much higher in the model group than in the control group (**Figure 9**), while Foxp3 expres-

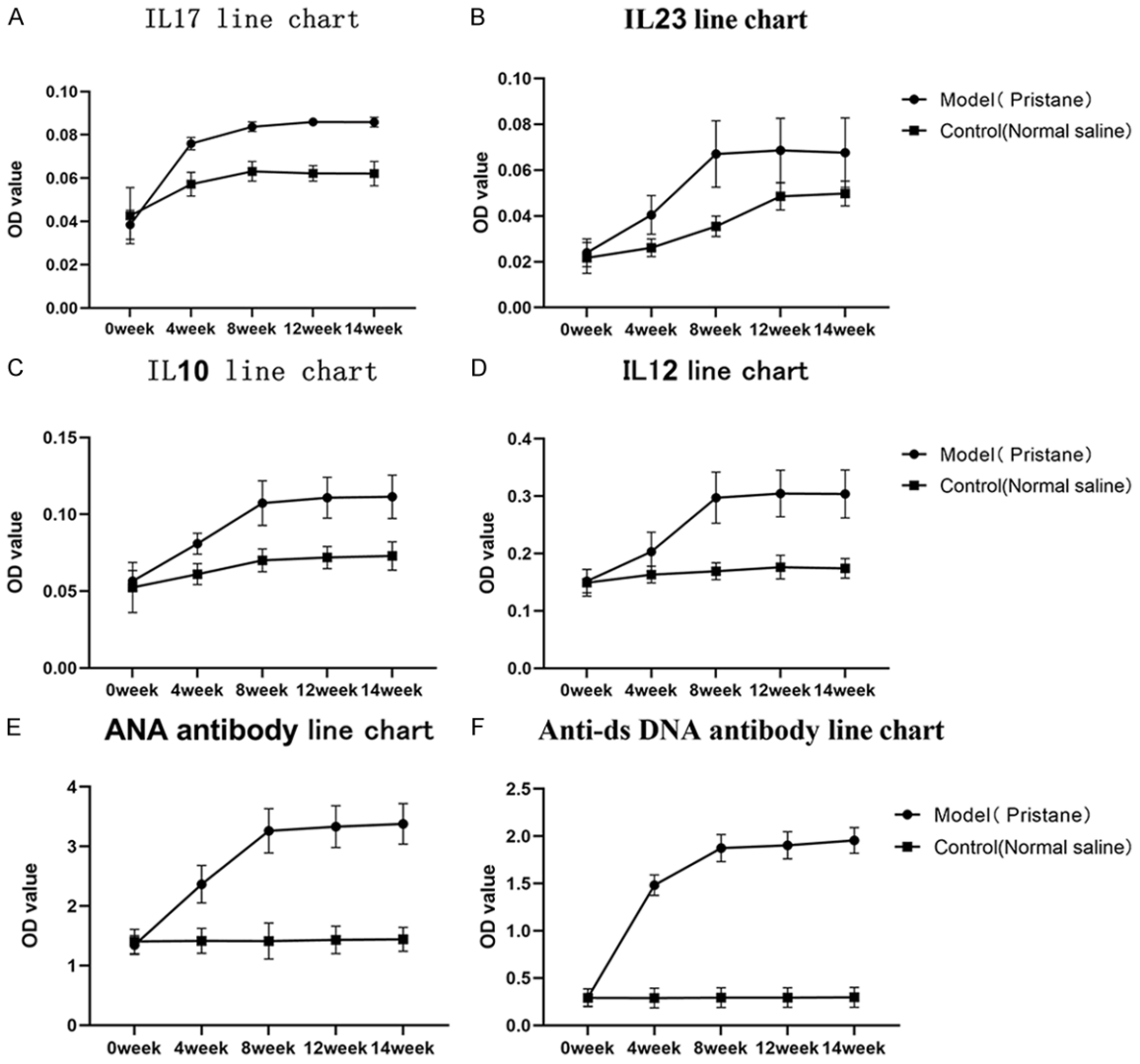


Figure 5. Expression of inflammatory factors after intraperitoneal injection.

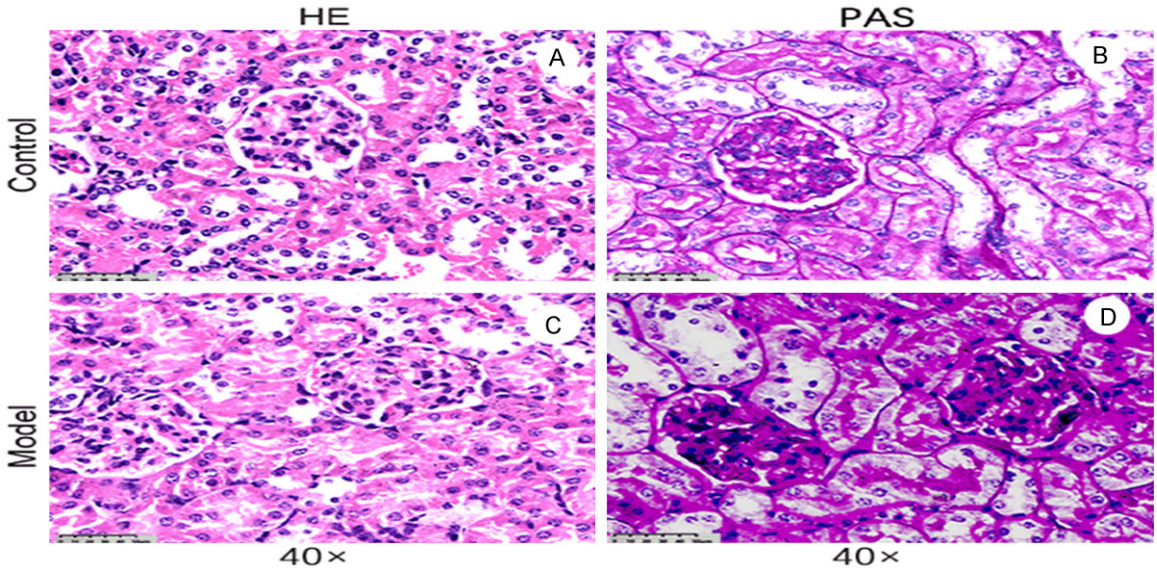
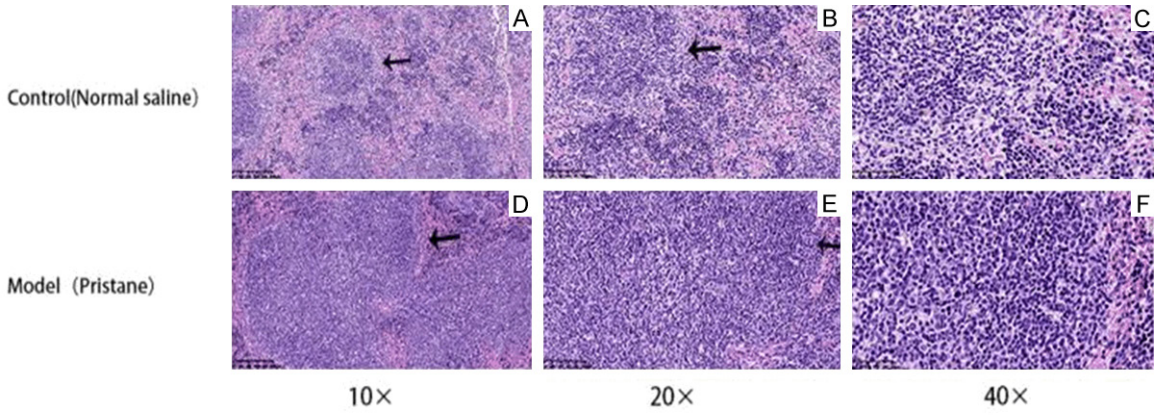


Figure 6. HE, Masson, and PAS staining of mouse kidneys.



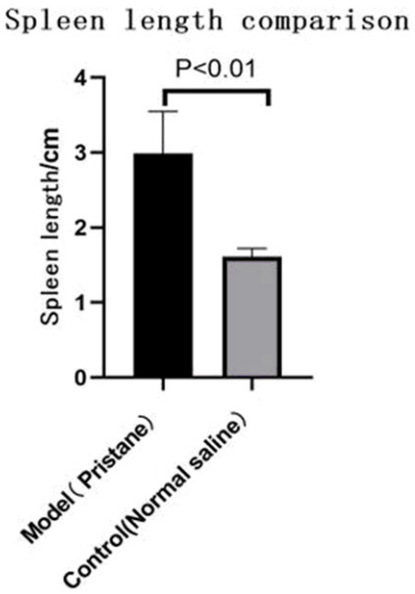


Figure 7. HE staining of mouse spleens and comparison of spleen length between the two groups.

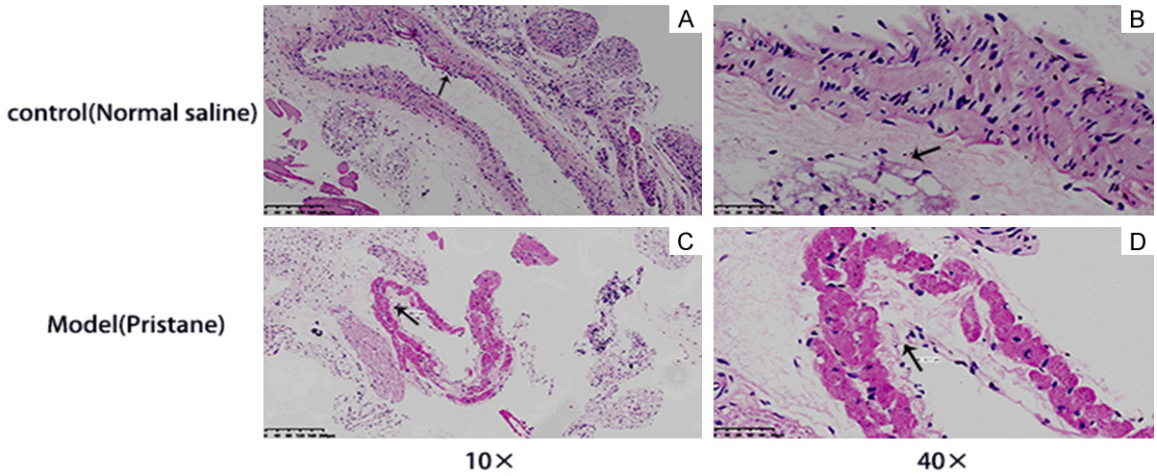


Figure 8. HE staining of mouse carotid arteries.

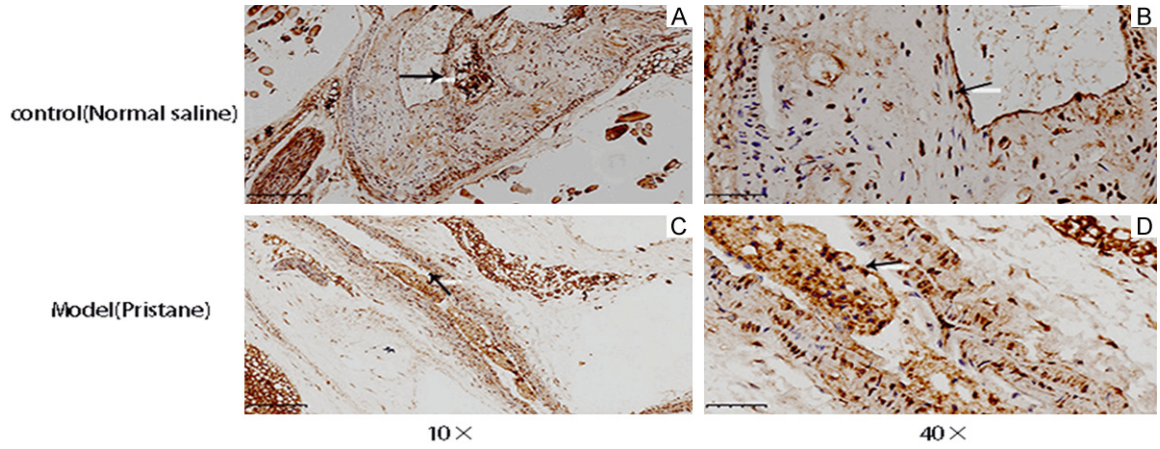


Figure 9. Immunohistochemistry showing IL17 expression in mouse carotid atherosclerotic plaques.

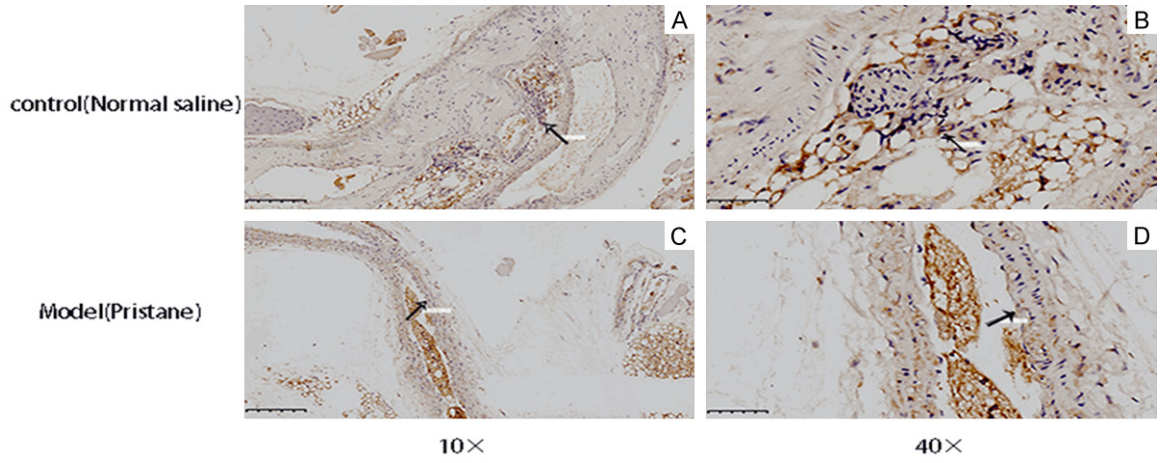


Figure 10. Immunohistochemistry showing Foxp3 expression in mouse carotid atherosclerotic plaques.

sion was lower in the model group than in the control group (**Figure 10**).

Flow cytometry

The number of $CD4^+CD25^+$Treg and $CD4^+$ $IL17^+$Th17 cells in the peripheral blood of mice indicated that the expression levels of the inflammatory factor IL-17A in the peripheral blood of mice in the model group were higher than those in the control group, while the levels of FOXP3 were lower than those in the control group (**Figure 11**).

Discussion

Previous studies showed that the main causes of death in patients with SLE gradually changed from infection and severe kidney disease in the last century to CVD [9]. Patients with SLE were five to ten times more likely to develop CVD than their peers [10] and had a more than two times higher risk of myocardial infarction [11]. Atherosclerosis, the underlying cause of various CVDs, is not easily detected at the initial stage. However, in the follow-up of a prospective study, 32% of patients with SLE had a tendency to develop carotid atherosclerotic plaque, while in healthy controls the proportion was only 4% [12]. Moreover, the fatality rate of SLE complicated by AS has gradually increased in recent years [13, 14].

Studies have suggested that $LDLr^{-/-}$ mice could be successfully modeled by high-fat feeding after one intraperitoneal injection of Pristine reagent [15] , but there is currently no uniform standard for the construction of this model.

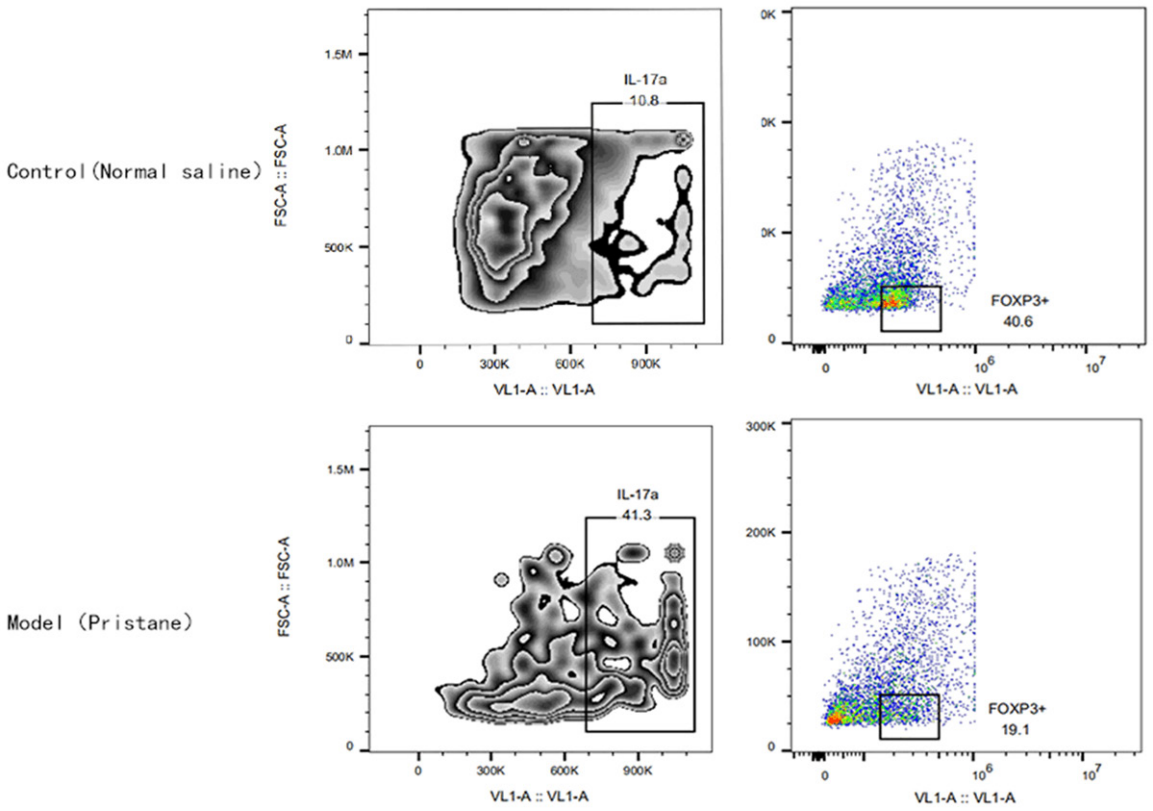


Figure 11. Flow cytometric diagram of the expression of inflammatory factors in mouse peripheral blood.

The results of this study showed that the control group, which was fed a high-fat diet alone, had increased TC, TG, and LDL-C levels, decreased HDL-C levels, and carotid atherosclerosis. These features are also observed in patients with SLE, who have a more significant lipid profile that promotes AS [16]. On the other hand, in addition to presenting hyperlipidemia and carotid atherosclerosis, the mice in the model group, which were injected with Pristine reagent into the abdominal cavity, were positive for specific ANA and anti-ds-DNA antibodies, and the inflammatory factors IL-17, IL-23, IL-12, and IL-10 showed an increasing trend with time. Previous studies of our research group suggested that Treg and Th17 cells may be in a balanced state under normal conditions. Meanwhile, the balance between these cells is disturbed in patients with SLE-AS, who present higher levels of Th17 cells [17]. This was mainly measured by the expression of IL-17, which is secreted by Th17 cells, and Foxp3, which is secreted by Treg cells. Therefore, Th17/Treg imbalance may be one of the causes of the high incidence of AS in patients with SLE. In addition, studies have suggested that IL-23, as a pro-inflammatory factor that forms the IL-23/IL-17 axis with IL-17, induces an inflammatory response and promotes the formation of atherosclerotic plaques (8). Additionally, IL-12, another pro-inflammatory factor, can enhance the inflammatory effects of IL-23 and IL-17, thereby inducing the occurrence of SLE complicated by AS. Furthermore, in our study, there was a large number of calcifications attached to the abdominal and thoracic organs in the model group, the splenomegaly was extremely obvious, the renal tubules were arranged in a disorderly manner, and the glomeruli were fibrotic to a certain extent. Flow cytometry suggested that IL-17 expression was higher in the model group, while Foxp3 expression was lower, and that there was a Th17/Treg imbalance, in comparison to the control group.

Our results were consistent with the clinical manifestations of SLE complicated by AS, confirming that the modeling was successful. The establishment of this stable and reliable animal model can provide an important experimental and scientific basis for studying the mechanism of this disease and creating effective prevention and treatment schemes.

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Disclosure of conflict of interest

None.

Abbreviations

ANA, antinuclear antibody; anti-dsDNA, anti-double-stranded DNA antibody; AS, atherosclerosis; CVD, cardiovascular diseases; HDL-C, high-density lipoprotein cholesterol; HE, hematoxylin-eosin; IgG, immunoglobulin G; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; PAS, periodic acid-Schiff; SLE, systemic lupus erythematosus; TBS, Tris-buffered saline; TC, total cholesterol; TG, triglycerides; Th17, T helper 17; Treg, regulatory T.

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References

[1] McMahon M and Skaggs B. Pathogenesis and treatment of atherosclerosis in lupus. Rheum Dis Clin North Am 2014; 40: 475-495.

[2] Iaccarino L, Bettio S, Zen M, Nalotto L, Gatto M, Ramonda R, Punzi L and Doria A. Premature coronary heart disease in SLE: can we prevent progression? Lupus 2013; 22: 1232-1242.

[3] Sharma SK, Rathi M, Sahoo S, Prakash M, Dhir V and Singh S. Assessment of premature atherosclerosis in systemic lupus erythematosus patients with and without nephritis. Lupus 2016; 25: 525-531.

[4] Fei Y, Shi X, Gan F, Li X, Zhang W, Li M, Hou Y, Zhang X, Zhao Y, Zeng X and Zhang F. Death causes and pathogens analysis of systemic lupus erythematosus during the past 26 years. Clin Rheumatol 2014; 33: 57-63.

[5] Zhu M, Mo H, Li D, Luo X and Zhang L. Th17/Treg imbalance induced by increased incidence of atherosclerosis in patients with systemic lupus erythematosus (SLE). Clin Rheumatol 2013; 32: 1045-1052.

[6] Ma J, Yu J, Tao X, Cai L, Wang J and Zheng SG. The imbalance between regulatory and IL-17-secreting CD4+ T cells in lupus patients. Clin Rheumatol 2010; 29: 1251-1258.

[7] Yang J, Yang X, Zou H and Li M. Oxidative stress and treg and Th17 dysfunction in systemic lupus erythematosus. Oxid Med Cell Longev 2016; 2016: 2526174.

[8] Abbas A, Gregersen I, Holm S, Daissormont I, Bjerkeli V, Krohg-Sorensen K, Skagen KR, Dahl TB, Russell D, Almas T, Bundgaard D, Alteheld LH, Rashidi A, Dahl CP, Michelsen AE, Biessen EA, Aukrust P, Halvorsen B and Skjelland M. Interleukin 23 levels are increased in carotid atherosclerosis: possible role for the interleukin 23/interleukin 17 axis. Stroke 2015; 46: 793-799.

[9] Andrades C, Fuego C, Manrique-Arija S and Fernandez-Nebro A. Management of cardiovascular risk in systemic lupus erythematosus: a systematic review. Lupus 2017; 26: 1407-1419.

[10] Giannelou M and Mavragani CP. Cardiovascular disease in systemic lupus erythematosus: a comprehensive update. J Autoimmun 2017; 82: 1-12.

[11] Avina-Zubieta JA, To F, Vostretsova K, De Vera M, Sayre EC and Esdaile JM. Risk of myocardial infarction and stroke in newly diagnosed systemic lupus erythematosus: a general population-based study. Arthritis Care Res (Hoboken) 2017; 69: 849-856.

[12] Baragetti A, Ramirez GA, Magnoni M, Garlaschelli K, Grigore L, Berteotti M, Scotti I, Bozzolo E, Berti A, Camici PG, Catapano AL, Manfredi AA, Ammirati E and Norata GD. Disease trends over time and CD4+ CCR5+ T-cells expansion predict carotid atherosclerosis development in patients with systemic lupus erythematosus. Nutr Metab Cardiovasc Dis 2018; 28: 53-63.

[13] Croca S and Rahman A. Atherosclerosis in systemic lupus erythematosus. Best Pract Res Clin Rheumatol 2017; 31: 364-372.

[14] Chen X and Xu G. Intravenous thrombolysis in SLE-related stroke: a case report and literature review. Neurol Sci 2018; 39: 155-159.

[15] Braun N, Wade NS, Wakeland EK and Major AS. Accelerated atherosclerosis is independent of feeding high fat diet in systemic lupus erythematosus-susceptible LDLr(-/-) mice. Lupus 2008; 17: 1070-1078.

[16] Szabo MZ, Szodoray P and Kiss E. Dyslipidemia in systemic lupus erythematosus. Immunol Res 2017; 65: 543-550.

[17] Zhu MY, Lin D, Liu J and Mo HY. Artesunate interfere in modulation of Foxp3 expression in synovial cells in collagen-induced arthritis rats. Chin J Integr Med 2016; [Epub ahead of print].