Original Article
APA, Anti-GPIIb/IIIa antibody and AECA associated with thrombosis in patients with SLE or pAPS and induced HUVEC apoptosis

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Abstract: Autoantibodies play important roles in connective tissue diseases (CTDs), like SLE (systemic lupus erythematosus) or its secondary disease pAPS (primary antiphospholipid syndrome) complicated by thromboembolism (TE). It is speculated that several auto-antibodies, containing antiphospholipid antibody (APA), Anti-GPIIb/IIIa antibody and anti-endothelial cell antibody (AECA) may be related with the formation of TE, while their role in the pathogenesis remains unknown. In this study, we aimed to analyze the correlation between the above antibodies and two CTDs (SLE and pAPS), and further to explore the possible antibody-mediated mechanisms. In this study, 238 SLE patients and 18 pAPS patients were enrolled. All the patients were clinically analyzed firstly and thus the sites with active TE were determined. Besides, sera from all the patients and healthy controls were measured for IgG and IgM-APA, GPIIb/IIIa or AECA by cellular sandwich enzyme-linked immunosorbent (ELISA) and indirect immunofluorescence assays. After statistical analysis, results showed that DAI (disease activity index) and TE were associated with the antibodies. Moreover, IgM-AECA or APA were extracted from sera of SLE patients (SLE-IgM-AECA or APA), and added to HUVEC cell culture medium. Cell proliferation was assayed by MTT kit. Flow cytometry and fluorescence staining analysis were performed to observe cell apoptosis. Results revealed that AECA and APA inhibited cell viability and caused apoptotic of HUVEC, respectively. Taken together, APA, GPIIb/IIIa and AECA might induce the occurrence of TE in SLE and pAPS patients. This study contributed to promote the early treatment of CTDs patients and reduce complications and death.

Keywords: Connective tissue diseases, APA, AECA, apoptosis, TE (thromboembolism)

Introduction

Connective tissue diseases (CTDs) are actually a group of medical diseases and immune-mediated and inflammatory disorders [1]. A connective tissue disease (CTD) can be any disease which has the connective tissues of the body as a primary target of pathology. The connective tissues are composed of two major structural protein molecules, elastin and collagen, and in patients with CTD, it is common for the two major structural protein molecules to become injured by inflammation [2]. Because of the immune system which is directed against one's own body tissues (autoimmunity), many CTDs feature abnormal immune system activity with inflammation in tissues [2, 3]. Thromboembolism (TE) events can occur in many CTDs, such as systemic lupus erythematosus (SLE) and antiphospholipid antibody syndrome (APS) [4]. SLE, an inflammation of the connective tissues, can afflict every organ system [5, 6]. It occurs up to 9 times more common in women than men and it can be aggravated by sunlight [7]. There are reports that more than 90% of SLE patients have endometrial thickening and glass-like lesions, with nearly 10% of SLE patients suffering cerebral infarction, suggesting that the SLE patients have been concurrent with vasculitis or primary TE [8]. The annual incidence of TE in SLE patients is 26.8%-51.9% [9]. APS, also known as antiphospholipid-thrombosis syndrome (APL-T) or Hughes syndrome, is recently discovered as a non-organ-specific autoimmune disease [10, 11]. APS is secondary to a variety of diseases, the main of which are

Table 1. Basic information of CTDs

Patients	Number	Age (year)	Duration (year)	Male to female	TE (%)
SLE	238	34.6±12.7	4.9±5.2	1:8.7	38 (15.9)
pAPS	18	34.6±12.7	4.9±5.2	1:8.7	18 (100)

Table 2A. Locations of TE in Blood vessel of SLE and pAPS patients

Cases	Blood vessel		
	Artery	Artery & Vein	Vein
SLE	19	9	10
pAPS	11	3	4
Total	49	17	20

Table 2B. Locations of TE in organs of SLE and pAPS patients

Cases	Organs								
	a	b	c	d	e	f	g	h	i
SLE	15	13	9	4	4	1	2	4	1
pAPS	4	7	2	0	3	2	0	6	2
Total	26	27	15	5	10	6	2	10	8

a. Limbs arteries to veins; b. Central nervous system; c. Lung; d. Kidney; e, Coronary artery; f. Retinal arteriovenous; g. Caput femoris; h. Placenta; i. superior and inferior vena cava.

rheumatism, such as SLE and other CTDs [12, 13]. Also, Hughes suggests that APS is a new isoform of SLE, and is closely related to the formation of TE in SLE [14-16]. In about 1/3 SLE patients, APA (anti-phospholipid antibodies, also abbreviated APL, APLA) can be detected, which contain APT (anti-prothrombin antibodies), ACA (anti-cardiolipin antibodies), LA (lupus anti-coagulant) and β_2-glycoprotein I (β_2-GPI) [17]. AECA (anti-endothelial cell antibodies) plays an important role in CTDs-induced TE, and it can be detected in 46%-80% SLE patients [18]. Research has shown that, AECA has a high positive rate in systemic vasculitis. The clinical manifestations, disease activity and prognosis of systemic vasculitis have a great relevance with the titer of AECA [19].

Recently, the specific causes of most CTDs have been unknown. In this study, we analyzed the correlation between the autoantibodies: APA (including APT, ACA, LA and anti-β2GP1 antibodies), anti-GPIIb/IIIa antibody and AECA antibody and the two CTDs (SLE and pAPS) concurrent with TE. By detecting these antibodies,

we comprehensively analyzed the high-risk group of CTDs concurrent with TE and keep the patients away from TE, especially TE in the life-threatening important organs. Further, in vitro studies, we explored if APA and AECA antibody can induce endothelial cell apoptosis, and thus laid the foundation for the possible mechanisms of antibody-mediated thrombosis.

Materials and methods

Source of patients

Patients enrolled in this study were from the patients being diagnosed or treated from January 1998 to October 2014 in Beijing Union Medical College Hospital and Gong Li Hospital, Shanghai Pudong. All patients, containing 238 cases of SLE patients and 18 cases of pAPS (**Table 1**), owned a complete clinical history data and serum bank information. The determined SLE patients meet the diagnostic criteria of the American Rheumatism Association in 1982, with pAPS patients in line with the classification criteria proposed by Wilson in 1998. Normal serum of 100 healthy blood donors was from the blood bank.

Detection of thromboembolism (TE) locations in SLE and pAPS patients

In order to explore the relationship between CTDs and TE, the locations of TE in blood vessel (**Table 2A**) and organs (**Table 2B**) of SLE and pAPS patients were detected. Among them, TE in limbs arteries to veins, kidney and superior & inferior vena cava were detected by using angiography; TE in central nervous system were detected by MRI (Magnetic Resonance Imaging) and Skull CT; TE in lung were detected by V/Q (Ventilation/perfusion), CTV (color television) and CTPA (Computed Tomography Pulmonary Angiography); TE in coronary artery was detected by MRA (Magnetic Resonance Angiography) and electrocardiogram; TE in retinal arteriovenous was detected by fundus examination; TE in caput femoris was detected by MRI; TE in Placenta was detected by B ultrasonic.

Detection of APA, anti-GPIIb/IIIa and AECA in SLE and APS patients

Antibodies including APA (contains ACA, LA, APT and anti-β2GP1 antibodies) (17) anti-GPI-

Table 3. Positive rates of APA, anti-GPIIb/IIIa and AECA in SLE and APS patients

Antibodies	SLE patients (%) (238)	pAPS patients (%) (18)	Normal (%)
ACL	37.8	88.9	6.0
APT	22.3	27.8	4.0
anti-β2-GP1	20.2	27.8	2.0
LA	11.8	16.7	0.0
anti-GPIIb/IIIa	26.9	44.4	9.0
AECA	41.5	44.4	5.0

Ib/IIIa, AECA, GAPDH (glycer aldehyde 3phosphate dehydrogenase) and HRP (horseradish peroxidase) conjugated secondary antibodies were pursed from Abcam (Cambridge, UK). Intracellular ACA, APT, anti-β2GP1 and anti-GPIIb/IIIa levels of all the enrolled SLE and APS patients were determined using enzyme-linked immunosorbent assay (Elisa) according to the instructions of manufacture, with the Elisa kit pursed from JRDun Biotechnology, Co., Ltd., Shanghai, China. In addition, LA levels were detected by Bioclot LA kit (Biopool, USA). AECA levels was detected by Elisa and indirect immunofluorescence assay according to the manufacturer's instructions, with the kits obtained from JRDun and German medical laboratory diagnosis co. and LTD, respectively. The optical density of each kind of antibody in each well was detected within 30 min by a microplate reader at 450 nm.

Purification and determination of IgG antibody in the serum of patients

According to the above detection results of positive rates of APA and TE positions in SLE patients, one SLE patient with pulmonary embolism carrying APA (SLE-APA) was selected for the following study. Obtained the serum from the selected patient as a treated group with the normal serum as a control. Purified the IgG antibodies from the serum by using affinity chromatography with protein G (GE Healthcare, Shanghai, China). Then SLE-APA-IgG and normal-IgG were obtained and its concentration was determined by Bradford method with Bradford Protein Quantification Kit pursed from YESEN Bio. Shanghai, China.

Culture and identification of human umbilical vein endothelial cells (HUVECs)

Under aseptic conditions, HUVECs were isolated from a fresh umbilical cord, which was obtained from a healthy caesarean neonate in the Department of Gynecology and Obstetrics of Gong Li Hospital, Shanghai Pudong. HUVECs were incubated in M-199 medium (Gibco Bio. Shanghai, China), planted in the 25 cm^2 culture flask, and the incubator (Thermo Fisher Scientific Inc., Waltham, MA, USA) was set to 37°C, 5% CO_2 and 100% humidity. Medium was replaced once every other day. After 5 days, HUVECs were digested by 0.05% trypsin and 0.025% EDTA (Trypsin Bio., Shanghai, China), subcultured till the fourth generation and identified by immunohistochemistry assay.

Detection of HUVECs proliferation

The MTT assay kit (APPLYGEN, Beijing, China) was used to assess the function of IgG antibodies from the selected SLE serum on cell growth and proliferation. Briefly, HUVECs in logarithmic growth-phase were collected, and 1×10^4 cells/well was dispensed into 96-well culture plates with 100 μL culture medium. After incubation for 24 hours, different concentrations of the obtained IgG (1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml) were put in different wells. Each of the above concentrations IgG was considered as one treated group and IgG obtained from the normal serum was regarded as the control. Every treated or control group contained 3 parallel wells. At the continued incubation for 72 hours, cell viability was detected by MMT according to the instructions of manufacturer. The optical density (OD) at wavelength 570 nm was determined for the supernatant of each well using the plate reader Multiskan EX (Thermo Fisher Scientific Inc., Waltham, MA, USA). All experiments were performed at least three times.

Detection of HUVECs apoptosis

In order to evaluate the effects of SLE-IgG-APA on HUVECs, HUVECs apoptosis was identified by using Hoechst 33342/PI fluorescence staining kit (LEAGENE, Beijing, China). In brief, cell culture was collected into a sterile centrifuge tube and trypsinized. Cell suspension was obtained, centrifuged at 4°C × 1000 g for 5 mins and the supernatant were discarded, remaining about 50 μL cell culture. Cells were then resuspended by 1 mL pre-cooled 1 × PBS (Sangon Biotech), centrifuged again at 4°C × 1000 g for 5 mins, and the supernatant were discarded. Dispersed cells by hitting the centrifuge tube gently. Approximately $(0.1-1) \times 10^6$ cells were collected and resuspended by 0.9

Table 4. APA, anti-GPIIb/IIIa and AECA in SLE and pAPS patients associated with TE

Antibodies	SLE (238)		pAPS (18)	
	With TE (%) (38)	Without TE (%) (200)	With TE (%) (200)	Without TE (%) (18)
ACL	78.9*	30.0*	88.9	0
APT	25.3	21.5	27.8	0
anti-β2-GP1	31.6	18.0	27.8	0
LA	36.8*	7.0*	16.7	0
anti-GPIIb/IIIa	36.8	25.2	44.4	0
AECA	55.3	42.9	77.8	0

*$P<0.005$ (*P* value refers to chi-square test of the antibodies' positive rates between with and without TE in CTDs patients).

Table 5. APA, anti-GPIIb/IIIa and AECA in SLE and pAPS patients associated with Arteriovenous TE

Antibodies	CTDs patients (SLE and pAPS) with TE			
	Artery (42)	Vein (26)	Artery & Vein (12)	*P* value
ACL (36)	33 (91.6%)*	15 (41.6%)	12 (100%)	<0.005
APT (11)	9 (81.8%)	8 (72.7%)#	6 (50.0%)	<0.05
anti-β2-GP1 (17)	12 (70.5%)	7 (41.3%)	2 (16.7%)	
LA (17)	16 (94.2%)#	6 (23.5%)	5 (41.7%)	<0.05
anti-GPIIb/IIIa (22)	20 (90.9%)#	9 (22.7%)	7 (58.3%)	<0.05
AECA (30)	23 (54.8%)*	11 (42.3%)#	4 (33.3)	<0.005

#$P<0.05$, *$P<0.005$ (*P* value refers to chi-square test of the antibodies' positive rates associated with Artery and Vein in CTDs patients with TE).

mL cell stain buffer (2 × cell stain buffer and sterile deionized water mixed in equal proportions). Subsequently, added 5 μL Hoechst 33342 and 5 μL PI stain, mixed gently and incubated at 4°C for 20-30 min. After staining, cells were washed once by 1 × PBS, and results were observed under fluorescent microscope.

Statistical analysis

Data were counted with values expressed as mean ± SD in **Table 1**. All statistical analyses were conducted by T test in **Tables 4-7**. In all statistical comparisons, P<0.05 was considered to be statistically significant.

Results

Distribution of TE (thromboembolism) in SLE and pAPS

All the enrolled CTDs patients contained 238 SLE patients and 18 pAPS patients. Among them, 38 SLE patients were complicated by TE,

accounting for 15.9%; while all the pAPS patients were complicated by TE, accounting for 100% (**Table 1**). Concurrent TE existed in various parts of the two CTDs. It could be arteriovenous existing simultaneously (**Table 2A**) and multiple organs existing simultaneously. In SLE and pAPS patients, it can be detected that one patient could be suffering from arteriovenous TE. Multi-organs were complicated by TE, particularly involving several vital organs, like brain, lungs and heart. Incidences of TE in various common organs were as follows: Central nervous system (31.3%), Limbs arteries to veins (30.21%), Lung (17.4%), Coronary artery (11.6%), Placenta (11.6%), superior and inferior vena cava (9.3%), Kidney (5.8%), Retinal arteriovenous (6.9%), Caput femoris (2.3%) (**Table 2B**). Ten cases of SLE and pAPS patients combined with TE at lung and deep vein of lower limb. Two cases of pAPS patients suffering from TE at brain, heart, fundus oculi and deep vein of lower limb.

Relationship between antibodies and the two CTDs

According to the results of the previous detection, positive rates of APA, anti-GPIIb/IIIa and AECA in SLE and pAPS patients were calculated. As shown in **Table 3**, positive rates of all the antibodies SLE and pAPS patients were much higher than in normal (0%-9%). Among APA's three kind of antibodies, positive rates of ACL ran first, accounting for 37.8% in SLE and 88.9% in pAPS, with APT accounting for 22.3% in SLE and 27.8% in pAPS, and anti-β2-GP1 accounting for 20.2% in SLE and 27.8% in pAPS. Anti-GPIIb/IIIa and AECA also were detected in SLE and pAPS patients, the positive rates were 26.9% and 44.4% respectively for Anti-GPIIb/IIIa, and 41.5% and 44.4% respectively for AECA.

Relationship between antibodies and the two CTDs with TE

In order to explore the relationship between antibodies and the two CTDs, (SLE and pAPS),

Table 6. Activity indicators of SLE associated with APA, anti-GPIIb/IIIa and AECA

Activity indicators of SLE	APA				Anti-GPIIb/IIIa (71)	AECA (99)
	ACL (108)	anti-β2-GP1 (53)	APT (52)	LA (31)		
C$_3$ <60 U/L	45/92	27/92	17/92	15/92	24/92	35/92
PASP >30 mmHg	10/10*	4/10	4/10	2/10	3/10	3/10
γ-globulin >23.5%	1/39	3/39	3/39	0/39	3/39	5/39
ALB <3.5 g/l	18/36	10/36	11/36	10/36#	13/36	19/36
Plt <0.1 million/mm^3	65/92*	24/92	19/92	22/92*	41/92*	35/92
anti-DsDNA >20%	24/56	16/56	10/56	9/56	14/56	22/56
LDL >140 mg/dl	1/22	2/22	1/22	0/22	2/22	12/22
DA I>5	11/112	36/112*	27/112	23/112*	21/112	46/112

(PASP, Pulmonary arterial systolic pressure; ALB, Albumin; Plt, Platelets; DsDNA, Double-stranded deoxyribonucleic acid; LDL, Low Density Lipoprotein; DAI, disease activity index), *P<0.005, #P<0.05 (*P* value means SLE patients/Total patients with the corresponding antibody).

Table 7. Positive rates of activity indicators in SLE with and without TE

Activity indicators	With TE (%) n=38	Without TE (%) n=200	*P* value
C$_3$ <60 U/L	50.0	40.9	
PASP >30 mmHg	10.5	3.2	<0.05
γ-globulin >23.5%	21.1	12.3	
ALB <3.5 g/l	21.1	18.2	
Plt <0.1 million/mm^3	68.4	34.4	<0.005
anti-DsDNA >20%	26.3	25.9	
TC >70 mg/dl	26.3	12.3	<0.05
LDL >140 mg/dl	23.6	9.1	<0.005

PASP, Pulmonary arterial systolic pressure; ALB, Albumin; Plt, Platelets; DsDNA, Double-stranded deoxyribonucleic acid; TC, Total cholesterol; LDL, Low Density Lipoprotein.

positive rates of APA, anti-GPIIb/IIIa and AECA in SLE and pAPS with TE patients were calculated. As shown in **Table 4**, all pAPS patients were complicated with TE, and high positive rates of all the antibodies were detected. Positive rates of all the antibodies in SLE with TE patients were much higher than that without TE. Among all the statistical data, there were significant differences (P<0.005) for ACL and LA in SLE with and without TE patients.

Relationship between antibodies and the two CTDs with arteriovenous TE

Total 56 cases of SLE and pAPS patients were complicated with TE, and among these, arterial TE accounting for 42 cases (28 cases in SLE and 14 cases in pAPS) and venous TE accounting for 26 cases (19 cases in SLE and 7 cases in pAPS). Thus, there were 12 cases of patients suffering from arterial and venous TE simulta-

neously, with 9 cases in SLE and 3 cases in pAPS (**Table 2**). By analysis of this group of 56 cases of patients, we found that (**Table 5**) ACL (P=0.0001, OR=13.4), LA (P=0.029, OR=8.0) and anti-GPIIb/IIIa (P=0.027, OR= 5.5) were associated with arterial TE, APT was associated with venous TE (P<0.05), and AECA was associated with not only arterial (P=0.001, OR=4.21) but also venous TE (P=0.003, OR=1.45), with anti-β2-GP1 associated with neither arterial nor venous TE.

Relationship between antibodies and activity indicators of SLE

According to the statistical results in **Table 6**, ACL was associated with thrombocytopenia (P=0.000, OR=5.0) and pulmonary hypertension (P=0.0005, OR=4.8). LA was associated with thrombocytopenia (P=0.000, OR=4.3), DAI (P=0.003, OR=3.4) and low albumin (P=0.03, OR=2.8). Anti-β2-GP1 was associated with DAI (P=0.003, OR=2.6). While the rest antibodies (P>0.05) were found no association with the indexes.

Relationship between activity indicators of SLE and TE

As shown in **Table 7**, all the positive rates of activity indicators in SLE complicated with TE were higher than that without TE. In SLE with TE and without TE patients, it presented 10.5% vs 3.2% for pulmonary hypertension (P<0.05), 68.4% vs 34.4% for Thrombocytopenia (P<0.005); 26.3% vs 12.3% for hypercholesterolemia (P<0.05) and 23.6% vs 9.1% for high LDL (P<0.005). This suggested that the incidence of
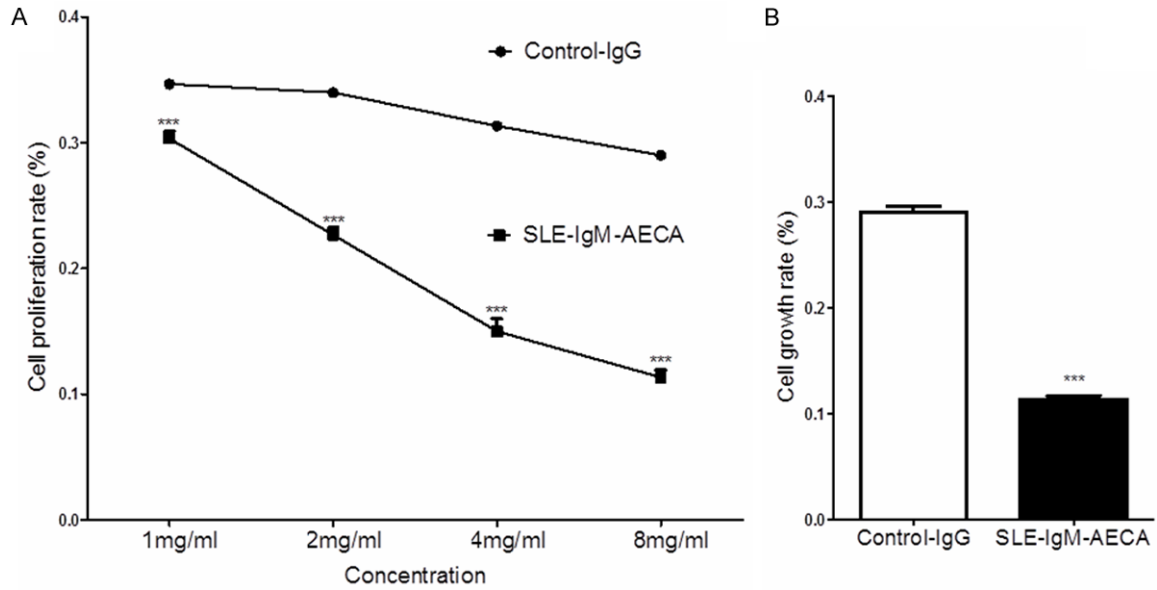
Figure 1. SLE-IgG-AECA inhibited HUVECs cell viability. A. Cell viability of *HUVECs* was weakened markedly in a dose-dependent manner. B. Cell proliferation rates of *HUVECs* in *SLE-IgG-AECA* group were significantly obstructed compared with that of the control. *n*=3, mean ± SD. ***P<0.001 *vs* control group.
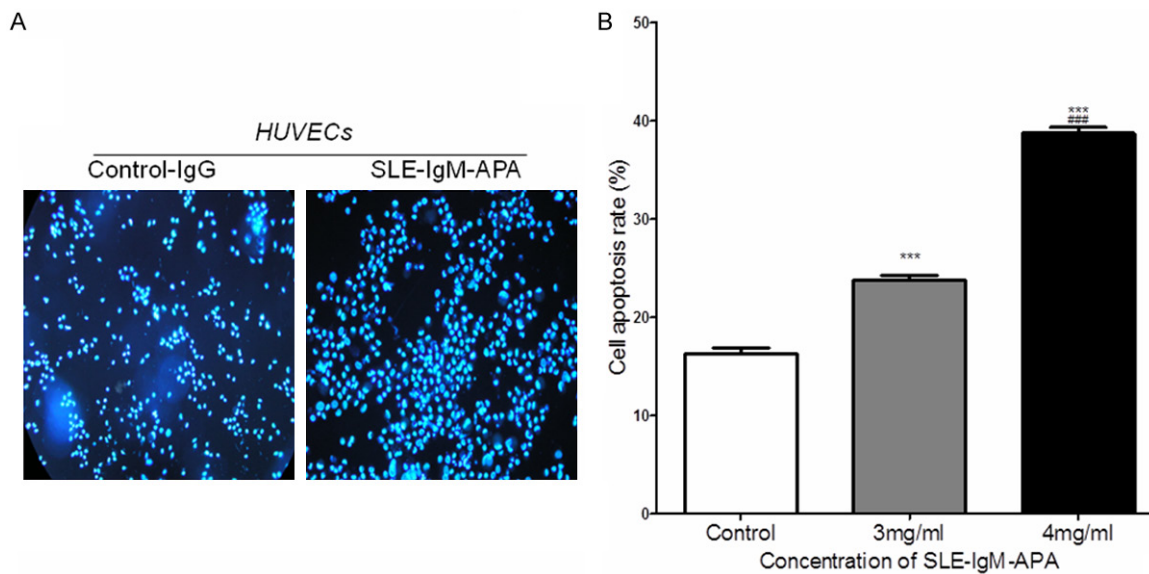

Figure 2. SLE-IgM-APA induced apoptosis of HUVECs. A. The number of apoptotic HUVECs in SLE-IgM-APA group was significantly greater than the control group. B. The apoptosis rates of HUVECs in control group, and groups treatment with 3 mg/ml or 4 mg/ml were successively increased. *n*=3, mean ± SD. ***P<0.001 *vs* control group.

all this activity indicators in SLE complicated by TE patients were significantly higher than those without TE. High levels of TC (Total Cholesterol) and LDL (Low Density Lipoprotein) may be risk indexes for TE in SLE patients, and thrombocytopenia might be the result. Pulmonary hypertension might be the reason or the result.

SLE-IgG-AECA inhibited HUVECs cell viability

Cell growth (OD/450 nm) and proliferation rates were measured by MMT kit. Results showed that in the *SLE-IgG-AECA* group, cell viability of *HUVECs* were weakened markedly in a dose-dependent manner (n=3, *P*<0.001) (**Figure 1A**).

On 8 mg/mL IgG groups, proliferation rates of HUVECs in *SLE-IgG-AECA* group were significantly obstructed compared with that of the control (n=3, *P*<0.001) (**Figure 1B**). Taken together, *SLE-IgG-AECA* inhibited growth and proliferation of *HUVECs*.

SLE-IgG-APA induced apoptosis of HUVECs

Results showed that after HUVECs were treated by 4 mg/ml SLE-IgM-APA, the number of apoptotic HUVECs in SLE-IgM-APA group were significantly greater than the control group (**Figure 2A**). Additionally, the apoptosis rates of HUVECs in control group, and groups treatment with 3 mg/ml or 4 mg/ml SLE-IgG-APA were (16.27±0.61)%, (23.75±0.53)%, (38.75± 0.58)%, respectively (n=3, P<0.01) (**Figure 2B**). This indicated that IgG-APA from sera of SLE patients induced apoptosis of HUVECs.

Discussion

In connective tissue diseases (CTDs), systemic lupus erythematosus (SLE) belongs to the group of diseases with high mortality rate [20]. The first five years of newly diagnosed SLE had recurrent activity, and the first death peak might appear in this stage. Currently, with the improvement of the level of treatment, the death caused by SLE was mainly concentrated in the second death peak. Infection, central nervous system lupus, kidney failure and cardiovascular disease could be the leading cause of death [21, 22]. Among these, the mortality rate from the second peak due to cardiovascular and TE became more and more prominent, and caused an increasing attention.

In SLE patients, it was reported that positive rate of antiphospholipid antibodies (APA) reached to 44% [22]. APA binding to endothelial cells and valves, caused platelet hypercoagulable state and injury of endothelial cell, and thus caused TE further [23]. In the current study, among the total enrolled 238 SLE patients, there were 38 cases of SLE complicated by TE, with the rate reaching 15.9%. While the TE occurrence rate of the 18 enrolled primary antiphospholipid antibody syndrome (pAPS) patients reached 100%. In the vessels with TE for SLE and APS patients, incidence of arterial thrombosis was much higher than venous thrombosis [24]. Thrombosis occurred in the central nervous system accounted for 31.3%,

in deep venous for 30.2%, in pulmonary vascular for 17.4% and in coronary for 11.6%. And there were 18 SLE patients, for whom, TE occurred in the pulmonary artery together with in the lower limb, suggesting that pulmonary vascular thrombosis from one hind was due to cardiovascular diseases, on the other hand it may be caused by deep vein thrombosis [25]. Therefore, actively administering treatment was needed for deep vein TE patients, and it was a key to keep deep vein thrombosis to shed off enter the lung artery effectively.

Cardiovascular disease could easily lead to sequelae and even death in patients, which arouse our attention. The reason why SLE patients were complicated by deep venous thrombosis may because SLE patients long-term used corticosteroids in a hypercoagulable state [26]. Besides, patients were at large in disease active period, during which immune complexes were increased, degradation was decreased, inflammatory mediators was released, and then all these caused inflammatory response, resulting in vascular injury. The above several factors caused thrombosis [27]. Disease activity index (DAI) of SLE patients with TE was 13.6±7.1, without TE was 9.7±6.1, between which there was a significant difference (T test, P<0.05) and for the former, patients were at large in disease active period. Further, in the analysis of the relationship between the occurrence of TE in patients and disease activity indicators, we found that pulmonary hypertension in SLE patients with TE accounted for 10.5%, without TE for 3.2%, and they had a significant correlation (T test, P<0.05). It suggested that SLE patients with pulmonary hypertension were prone to the occurrence of TE from one hand; on the other hand, the occurrence of TE in SLE patients was prone to pulmonary hypertension [28]. As for which were the causes or results, further observation was needed in the future.

In our studies, platelets in SLE patients with TE dropped to 68.4%, without TE to 34.4% (P<0.005), suggested that platelets were depleted significantly during the occurrence of TE. Recently, the role of platelets in TE formation caused growing attention. During the injury of endothelial cell or under the function of inflammatory mediators, platelets were activated and combined with the membrane glycoprotein re-

ceptor, causing platelet aggregation [28, 29]. Meanwhile, the platelet membrane glycoprotein receptor combined with fibrinogen promoted platelet aggregation further, which was very important in the formation of arterial thrombosis, especially in the vascular obstruction of cardiac-cerebral vascular disease [28]. In addition, cholesterol and low density lipoprotein in SLE patients with TE were increased (26.3% and 23.6%, respectively), compared with SLE patients without TE (12.3% and 9.1%, respectively), which had a significant difference (P< 0.05 and P<0.005). As we all know, increased cholesterol and low density lipoprotein in serum were significantly associated with atherosclerosis, which were risk factors of cardiovascular embolism forming [29]. Premature atherosclerosis was one of the main reasons caused SLE thrombosis/embolism, resulting in cardiovascular diseases such as myocardial infarction and cerebral infarction, even causing death [30]. So, it is suggested that for SLE associated with increased cholesterol and low density lipoprotein patients, appropriate treatments were essential to reduce the incidence of atherosclerosis. Other indicators: in SLE patients with or without TE, γ-globulin accounted for 21.2% and 12.3% respectively, albumin for 21.1% and 18.2%, complement for 50% and 40.9%, anti-DsDNA antibodies for 26.3% and 25.9%, suggesting that the indicators in SLE patients with TE were slightly higher than those without TE, with no significant difference statistically (P> 0.05). According to the reports, SLE patients with TE may be due to some of the following factors, such as vascular endothelial injury, APA-mediated TE, hypoalbuminemia, hyperglobulinemia or long-term using of hormones, which was consistent with our results that hyperglobulinemia patients were complicated by more TE [31, 32].

On the basis of the above clinical analysis, detection and analysis of APA (APT, ACA, β_2-GPI, LA), anti-GPIIb/IIIa and AECA in SLE and pAPS patients were performed. Prothrombin was the precursor protein of thrombin, and played an important role in balancing coagulation and anticoagulation of the body [33]. The reasons why APT was associated with the occurrence of venous thrombosis might because APT inhibited the release of thrombin-mediated endothelial prostacyclin, obstructed the activity of protein C, and might be a sera marker of CTDs,

especially SLE complicated by TE [34, 35]. For β_2-GPI, it can mediate APA combined with cell/phospholipid membrane, resulting in the expression of cell adhesion molecules increased and the scavenging of apoptosis promoted [36, 37], which has a great effect on the pathogenesis of APA [38, 39]. As for GPIIb/IIIa, it is a receptor of adhesion molecules and belongs to integrin family [40], and when it is combined with fibrinogen, bridging formation between platelets and platelet aggregation occur [41, 42]. In our study, the positive rates of APA, anti-GPIIb/IIIa and AECA in SLE and APS with TE patients were much higher than in that without TE patients. It was reported that coronary thrombosis relied on the activation and aggregation of platelets, which were mediated by GPIIb/IIIa receptor [43]. Pathogenesis of ischemic stroke depends on the platelet-rich TE, suggesting that anti-GPIIb/IIIa antibody was associated with arterial thrombosis [44]. This offered us a revelation, in the clinical detection, patients with positive ACL antibodies or anti-anti-GPIIb/IIIa antibody needed to be alert to the occurrence of TE. GPIIb/IIIa, as the main target structure of immune-related thrombocytopenia and antiplatelet antibodies, highly polymorphic anti-GPIIb/IIIa antibodies was significantly associated with immune thrombocytopenia and thrombocytopenia caused by APS [23, 45]. And it was consistent with our results. Additionally, through comparison analysis, we found that the correlation the correlation between DAI and the formation of TE was consistent with the correlation between DAI and the antibodies. Patients with TE also had thrombocytopenia and pulmonary hypertension, which meant the clinical SLE patients with positive ACL, anti-GPIIb/IIIa, and LA, complicated by pulmonary hypertension were more likely to suffer from TE [46, 47]. LA and hypoalbuminemia are the risk factors for TE, while it may also have LA positive patients with low albumin prone to suffer from TE. For AECA, positive rates of AECA in patients with TE were much higher than that without TE, which meant AECA was associated with TE, also similar results proved that it clearly associated with DAI [48]. It suggested that AECA and other antibodies we had explored could be used as indicators of disease activity in patients and make sense to patient treatment and prognosis [49]. Taken together, APA and its related antibodies and AECA had significant correlation with SLE, APS

and various vasculitis during their onset, disease activity and thrombosis occurrence. Early detection of these antibodies had important significance in TE prediction and the clinical diagnosis of the disease treatment.

In order to investigate the injured mechanism of the related positive antibodies from SLE patients function on endothelial cells further, cell proliferation and apoptosis assays were performed. And we proved that AECA from the sera of SLE patients inhibited HUVECs viability, moreover, APA from the sera of SLE induced cell apoptosis. When endothelial cells were injured, Annexin V combined with endothelial cell membrane, leading to cell membrane bilayer phospholipid endothelial valgus, causing the change of endothelial cell surface charge [50, 51]. And this change causes not only the change in the blood clotting mechanism of the endothelial cells, the enhanced procoagulant role and secretion of clotting factors, but also activation of platelets and platelet aggregation, which caused a blood coagulation and formation of TE [52]. Our study results were just consistent with this, and we proved APA could cause changes in endothelial cell membrane phospholipids, resulting in the formation of TE in patients [53].

Conclusion

In conclusion, APA and its related protein antibodies, together with AECA had a much closed relationship with the onset, disease activity, particularly TE in SLE, APS and other various vasculitis. By detecting these antibodies, active disease state of patients can be determined, and they could be used as indicators of clinical DAI, TE formation and also they could be used for determining patient outcomes. Additionally, by detecting these antibodies, we can predict the patients and their vascular thrombosis tend to suffer from TE, such as artery or vein. It will contribute to offer early treatment for patients and reduce complications or death.

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Disclosure of conflict of interest

None.

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