Increased Production of a Proliferation-inducing Ligand (APRIL) by Peripheral Blood Mononuclear Cells Is Associated with Antitopoisomerase I Antibody and More Severe Disease in Systemic Sclerosis

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ABSTRACT. ***Objective.*** A proliferation-inducing ligand (APRIL), a member of the tumor necrosis factor (TNF) family, plays a crucial role in the survival of peripheral B cells, and may contribute to the pathogenesis of systemic sclerosis (SSc) through upregulation of autoantibody production and maintenance of autoimmune phenomena. We evaluated the capacity of peripheral blood mononuclear cells from patients with SSc (SSc-PBMC) to produce APRIL; and investigated correlations between production of APRIL by SSc-PBMC and clinical and laboratory features of the disease.

Methods. PBMC from 20 patients with SSc and 14 healthy subjects were incubated in fetal calf serum-supplemented RPMI medium. APRIL levels were determined in cell culture supernatants by ELISA.

Results. PBMC from patients with SSc produced significantly more APRIL (961 ± 151 pg/ml/10^5 cells) than control PBMC (798 ± 219 pg/ml/10^5 cells; $p < 0.01$). In patients with SSc, increased production of APRIL was associated with the presence of diffuse skin involvement, scleroderma lung disease, peripheral vasculopathy, greater capillary damage on capillaroscopy, and presence of antitopoisomerase I (anti-topo I) antibodies. Multivariate regression analysis revealed anti-topo I antibodies as the only independent predictor of high production of APRIL by PBMC.

Conclusion. Production of APRIL is increased in SSc-PBMC and is associated with the presence of anti-topo I antibodies and more severe disease. Targeting the APRIL pathway might represent a therapeutic possibility for treatment of patients with SSc, in particular those with anti-topo I antibodies. (J Rheumatol First Release September 1 2010; doi:10.3899/jrheum.100454)

Key Indexing Terms:
SYSTEMIC SCLEROSIS SCLERODERMA APRIL PATHOGENESIS

A proliferation-inducing ligand (APRIL) is a member of the tumor necrosis factor (TNF) superfamily, and has been named for its capacity to stimulate cell growth and survival[1]. The most recognized properties of APRIL are its ability to stimulate B cell and plasma cell survival, and to regulate immunoglobulin class-switching. APRIL has therefore been implicated in the pathogenesis of B cell-derived malignancies as well as autoimmune diseases[2]. Elevated concentrations of APRIL were found in the peripheral blood of patients with systemic autoimmune diseases including systemic sclerosis (SSc)[3]. The cellular source of elevated APRIL levels in the blood of SSc patients has not been identified. APRIL is expressed by several cell types, including monocytes and lymphocytes, which are also major constituents of inflammatory infiltrates found in the skin and other organs early in the course of SSc[1,4].

We evaluated the ability of peripheral blood mononuclear cells (PBMC) from patients with SSc (SSc-PBMC) to secrete APRIL; and investigated associations between production of APRIL and clinical features of the disease.

MATERIALS AND METHODS

Twenty patients fulfilling American College of Rheumatology classification criteria for SSc[5] and/or the classification criteria for early SSc of LeRoy and Medsger[6] and 14 healthy controls were investigated (Table 1). Only patients who had not taken any immunosuppressive therapy or in whom immunosuppressive therapy had been stopped at least 6 months before blood collection were considered eligible.

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Table 1. Clinical characteristics of the patients with systemic sclerosis (SSc) and the control group.

Characteristic	SSc Patients, n = 20	Controls, n = 14
Female/male	17/3	11/3
Age, yrs, median (range)	50* (25–74)	39 (26–62)
Disease duration, yrs,† median (range)	2 (0–8)	
Duration of Raynaud's phenomenon, yrs, median (range)	5 (0.5–20)	
Early disease†† (%)	15 (75)	
ACR criteria fulfilled (%)	17 (85)	
dSSc/lSSc	7/13	
ANA-positive (%)	20 (100)	
Anti-topo I-positive (%)	12 (60)	
ACA-positive (%)	6 (30)	
Raynaud's phenomenon (%)	20 (100)	
SLD by HRCT/radiograph (%)	9 (45)	
Restrictive lung disease# (%)	2 (10)	
Pulmonary hypertension (%)	1 (5)	
Digital ulcers and/or pitting scars (%)	13 (65)	

Differences considered significant at p value < 0.05. * p = 0.07. † Calculated from first non-Raynaud's symptom attributable to SSc. †† Defined as shorter than 3 years in dSSc patients or shorter than 5 years duration in patients with ISSc. # Defined as forced vital capacity < 75% of predicted. ACA: anticentromere antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibodies; dSSc: diffuse cutaneous systemic sclerosis; HRCT: high resolution computed tomography; ISSc: limited cutaneous systemic sclerosis; SLD: scleroderma interstitial lung disease.

PBMC were isolated from whole blood from patients and controls and cultured as described[7]. Briefly, PBMC were incubated in fetal calf serum-supplemented RPMI medium at 37°C under 5% CO_2. Concentrations of APRIL in the PBMC cell culture supernatants were determined using commercial ELISA kits (Bender MedSystems GmbH, Vienna, Austria).

Patients with SSc were classified as having diffuse cutaneous SSc (dSSc) or limited cutaneous SSc (lSSc) based on criteria of LeRoy, *et al*[8].

Clinical and laboratory evaluations of patients with SSc were performed as described[7]. Capillaroscopic patterns were classified according to Carpentier and Maricq as the "slow" pattern, characterized by the preserved architecture of the vascular layout, or the "active" pattern, characterized by more severe damage and loss of capillaries[9].

For statistical analyses the ANOVA test, Mann-Whitney U test, the Spearman correlation test, and regression analyses were carried out, as appropriate.

The study protocol was approved by the local bioethics committee and all patients gave appropriate informed consent.

RESULTS

Specific concentrations of APRIL produced in controls, in the whole group of patients with SSc, and in SSc subgroups are shown in Table 2. SSc-PBMC produced significantly more APRIL compared with controls. Patients with dSSc produced more APRIL compared with those with lSSc or controls. Also, patients with scleroderma interstitial lung disease (SLD) produced more APRIL than patients without SLD or controls. Accordingly, the concentrations of APRIL correlated with the modified Rodnan skin scores (R = 0.78, p < 0.01), and correlated inversely with the forced vital capacity expressed as percentage of the predicted value (R = –0.52, p < 0.05).

In addition, production of APRIL was greater in patients with SSc who had the "active" capillaroscopic pattern compared with those with the "slow" pattern and controls; and greater in patients with peripheral vasculopathy (digital ulcers and/or pitting scars) than in patients with SSc who did not have vasculopathy.

Patients with anti-topoisomerase I autoantibodies (anti-topo I) produced more APRIL than those without anti-topo I and controls. There were no significant differences in the production of APRIL between patients with anticentromere autoantibodies (ACA) and controls. In contrast, patients without ACA produced greater amounts of APRIL than controls or patients with SSc who had ACA.

There was no correlation between APRIL levels and patients' ages or other disease measures, including disease duration, predicted DLCO values, erythrocyte sedimentation rate, and C-reactive protein concentration (data not shown).

Univariate analysis revealed significant correlations between production of APRIL and the extent of skin involvement, presence of SLD, "active" capillaroscopic pattern, and anti-topo I. Production of APRIL was negatively associated with the presence of ACA (Table 3). In multivariate analysis, including all measures identified by means of univariate analysis, production of APRIL correlated significantly only with the presence of anti-topo I (Table 3).

DISCUSSION

This is the first study showing that SSc-PBMC produce significantly more APRIL in a comparison with healthy controls. To date, only Matsushita, *et al*[3] have evaluated APRIL in SSc, showing that APRIL was elevated in serum from patients with SSc compared with controls. In addition, patients with SSc who had high serum APRIL levels had higher incidence of SLD compared with patients with SSc who had lower APRIL concentrations[3]. Our findings are in agreement with the results described by Matsushita, *et al*, in that we also found associations between increased production of APRIL and the presence of SLD.

However, unlike Matsushita, *et al*, we found significant associations between increased production of APRIL and greater skin involvement, greater vascular damage, and the specific immunological profile in SSc. As indicated by our multivariate analysis results, only the presence of anti-topo I was independently associated with increased production of APRIL by SSc-PBMC.

In patients with SSc, anti-topo I and ACA are mutually exclusive and identify different subsets of the disease. Anti-topo I has been well recognized as a marker of more severe disease, while ACA are associated with lSSc and good clinical prognosis[10,11,12]. It is therefore not surprising that increased production of APRIL showed associations with

Table 2. Production of APRIL by PBMC from healthy controls, the whole patient group and different subgroups of patients with systemic sclerosis (SSc). Patients with SSc were categorized according to the different clinical and immunological variables.

Category	Group	Concentration of APRIL in pg/ml/10^5 cells, mean ± SD	p, Between-group Comparisons
Healthy controls (HC)		798 ± 219	< 0.05 HC vs SSc
SSc patients		961 ± 151	
Skin involvement	Diffuse SSc (dSSc)	1071 ± 91	< 0.05 vs ISSc and HC
	Limited SSc (ISSc)	887 ± 138	NS vs HC
Scleroderma interstitial lung disease	Present	1076 ± 118	< 0.05 vs patients without SLD and HC
	Absent	864 ± 111	NS vs HC
Peripheral vasculopathy*	Present	1022 ± 146	< 0.05 vs patients without vasculopathy and HC
	Absent	850 ± 74	NS vs HC
Capillaroscopic pattern	"Active"	1050 ± 117	< 0.05 vs "slow" and HC
	"Slow"	908 ± 129	0.06 vs HC
Anti-topo I	Present	1052 ± 113	< 0.05 vs patients without anti-topo I and HC
	Absent	825 ± 78	NS vs HC
Anticentromere antibodies (ACA)	Present	843 ± 67	NS vs HC
	Absent	1011 ± 149	< 0.05 vs patients with ACA and HC

Differences considered significant at $p < 0.05$. * Defined as presence of digital ulcers and/or pitting scars. Anti-topo I: anti-topoisomerase I antibodies; dSSc: diffuse systemic sclerosis; ISSc: limited systemic sclerosis; NS: not significant; PBMC: peripheral blood mononuclear cells, SLD: scleroderma interstitial lung disease.

Table 3. Results of univariate and multivariate analyses regarding associations between production of APRIL and clinical and laboratory features of SSc.

Clinical or Laboratory Feature	Univariate Analysis		Multivariate Analysis	
	Corrected R^2	p	Beta Coefficient	p
Form of SSc (dSSc/ISSc)	0.35	0.009	0.51	> 0.05
Modified Rodnan skin score	0.48	0.002	0.40	> 0.05
Scleroderma interstitial lung disease	0.41	0.004	0.73	> 0.05
Forced vital capacity	0.12	0.1		
Capillaroscopic pattern ("slow" vs "active")	0.22	0.04	0.14	> 0.05
Peripheral vasculopathy*	0.17	0.06		
Anti-topo I antibody	0.52	0.001	0.85	0.01
Anticentromere antibody	0.38	0.007	0.49	> 0.05

Differences considered significant at $p < 0.05$. * Defined as presence of digital ulcers and/or pitting scars. dSSc: diffuse cutaneous systemic sclerosis; ISSc: limited cutaneous systemic sclerosis.

more severe disease, and that ACA-negative patients, the majority of whom had anti-topo I, produced more APRIL than did ACA-positive patients.

Since anti-topo I antibody was the only independent predictor for increased production of APRIL, and APRIL is known to stimulate B cell functions, it is tempting to hypothesize that APRIL might contribute to the development of SSc through stimulation of autoimmune phenomena. However, further studies are required to clarify the molecular mechanisms responsible for elevated APRIL production by the PBMC from anti-topo I-positive patients with SSc. Finally, associations revealed in our study may suggest that anti-topo I-positive patients might particularly benefit from therapies targeting APRIL pathways; and that evaluation of autoantibody status might be helpful in selecting patients with SSc for this kind of treatment. Therapies aimed at blocking APRIL pathways are being investigated in the setting of clinical trials in other autoimmune diseases[13,14].

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