Soluble CD154 Is Not Associated with Atherosclerosis in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Soluble CD154 (sCD154) is involved in the pathogenesis of systemic lupus erythematosus (SLE), as well as in the initiation and progression of atherosclerotic lesions. We determined the association of sCD154 with coronary calcium and carotid plaque at the baseline visit of the Lupus Atherosclerosis Prevention Study.

Methods. Serum samples were assayed for soluble CD154 by ELISA. Coronary calcium was measured by helical computed tomography. Carotid duplex was performed to measure carotid plaque.

Results. sCD154 was measured in 183 patients with SLE. Patients had a mean age of 48.8 ± 10.5 yrs, and 92% were female. Ethnicity included 61% Caucasian, 34% African American, 2% Asian, and 2% Hispanic. sCD154 was not associated with carotid plaque (p = 0.45) nor with coronary calcium (p = 0.43). Indeed, those with carotid plaque had a trend toward lower levels of sCD154 (474 ± 29.2 vs 526 ± 5 pg/ml; p = 0.45).

Conclusion. sCD154 is not associated with subclinical measures of atherosclerosis in SLE, including carotid plaque and coronary calcium. (First Release April 1 2007; J Rheumatol 2007;34:969–72)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS | CD154 | ATHEROSCLEROSIS
CORONARY CALCIFICATION | CAROTID PLAQUE

Soluble CD154 (sCD154), also called CD40L, is a member of the tumor necrosis factor family. It is expressed as a soluble cytokine and as a homotrimeric type II transmembrane protein on the surface of activated CD4-positive T lymphocytes[1]. sCD154 leads to B cell differentiation, proliferation[2], germinal center formation[3], and antibody isotype switching[4]. Endothelial cell expression of sCD154 is increased by other proinflammatory cytokines[5]. Abnormal or overexpression of sCD154 on CD4-positive T cells is important in the pathogenesis of systemic lupus erythematosus (SLE)[6,7].

The binding of sCD154 to its receptor, CD40, mediates several inflammatory responses that are important in atherosclerosis. Ligation of sCD154 triggers the release of chemoattractants overexpressed in human atheroma as well as the expression of leukocyte adhesion molecules[8-10]. Expression of tissue factor, an important prothrombotic component of the intraplaque lipid pool, is also strongly induced by sCD154 ligation[11,12]. Animal studies have shown that inhibition of sCD154 signaling in atherosclerosis-prone mice reduces the size and lipid content of aortic lesions[13]. sCD154 signaling also results in destabilization of atherosclerotic plaque, by inducing the expression of cytokines, chemokines, growth factors, and procoagulant factors in a variety of atheroma-associated cell types[14].

sCD154 concentrations have been shown to be a marker of unstable angina[15]. In a nested case-control study performed in the OPUS-TIMI16 trial, elevated sCD154 levels were associated with myocardial infarction, congestive heart failure, and death[16]. However, in the Dallas Heart Study, there was no association of sCD154 with carotid plaque[17].

Premature atherosclerosis is the major cause of mortality in SLE in developed countries[18,19]. sCD154 levels have been shown to be higher in patients with SLE than in the general population[20]. In one study of 26 SLE patients, sCD154 levels were associated with coronary calcium in SLE[21]. However, another study of 197 SLE patients found no association with carotid plaque[22]. We determined the association of sCD154 with coronary calcium, carotid plaque, and other cardiovascular risk factors at the baseline visit of the Lupus Atherosclerosis Prevention Study.

MATERIALS AND METHODS

A total of 200 patients with SLE were enrolled in the Lupus Atherosclerosis Prevention Study. The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board. All patients gave informed consent. Patients with a history of atherosclerotic event (angina or myocardial infarction) were excluded. Patients with a low density lipoprotein level of 190 mg/dl or triglyceride level > 500 mg/dl were also excluded. At the baseline visit, helical computed tomography (CT) (for coronary calcium score) and carotid duplex investigation (for carotid plaque score) were performed, as

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described below. Soluble CD154 levels were measured on stored baseline sera available from 183 patients. Disease activity was measured using the Physician Estimate of Activity (physician's global assessment on a 0 to 3 visual analog scale) and the SELENA SLEDAI (SLE Disease Activity Index)[23].

Image acquisition and evaluation. Carotid duplex was performed using high resolution linear transducers (5–10 MHz; Acuson 128XP, Hewlett Packard Image Point or ATL HDI 3000) in the General Clinical Research Center at Bayview Medical Center. Images were acquired of the distal common carotid arteries, carotid bulb, and proximal internal carotid arteries (ICA) in the sagittal plane. Doppler spectrum tracings were taken in the first 2 cm of the proximal ICA. If carotid plaque was identified, transverse images and measurements of plaque were performed.

Coronary calcium was assessed on helical CT with a Siemens Volume Zoom Scanner (Siemens, Malvern, PA, USA) using a 2.5 mm collimation and a slice width of 3 mm. Data were reloaded into a Siemens Leonardo workstation, using the Siemens calcium scoring software. The software calculated a calcium score for coronary calcification[24].

Measurement of sCD154. Stored serum samples from the baseline study visit (n = 183) were assayed for sCD154 by ELISA (R&D Systems, Minneapolis, MN, USA). Capture antibody was immobilized onto enzyme immunoassay plates overnight, and then blocked with 10% fetal calf serum in phosphate buffered saline. Sera diluted 1:5 in fetal calf serum blocking buffer were added in duplicate and incubated 2 h. sCD154 was detected with a biotinylated detection antibody, followed by streptavidin-horseradish peroxidase. Tetramethyl benzidine (KPL Laboratories, Gaithersburg, MD, USA) was used as the colorimetric substrate.

Standards were generated using pooled human serum spiked with recombinant sCD154. The resulting curves were fit using a 4-parameter logistic curve-fitting algorithm. Measurement of sCD154 in lupus patients by capture ELISA was first reported by Vakkalanka, *et al*[20] and by Kato, *et al*[25], and has been widely used[26,27] to investigate sCD154 expression in SLE.

Cardiovascular risk factors. Cardiovascular risk factors that were assessed at baseline included body mass index, hypertension, use of antihypertensive medication, diabetes mellitus, and smoking. Fibrinogen, lipoprotein(a), and high sensitivity-C-reactive protein (CRP) levels were available from the Hopkins Lupus Cohort database.

Statistical analysis. Statistical analysis was performed using JMP (v5.0.1, SAS Institute, Cary, NC, USA). A p value of 0.05 was taken as statistically significant. One-way ANOVA was performed for normally distributed variables.

RESULTS

Data were obtained on 183 SLE subjects; 92% of patients were female; 61% were Caucasian, 34% African American, 2% Asian, 2% Hispanic, and 1% other ethnicity. The mean age was 44.3 ± 11.4 years. The level of soluble CD154 ranged from 5.47 to 2717.46 pg/ml, with a mean level of 483.2 ± 346 pg/ml. Coronary calcification was found in 43% of patients and carotid plaque in 58%. There were no demographic or traditional cardiovascular risk factor associates of soluble CD154 (Table 1). The association of hs-CRP with sCD154 failed to meet statistical significance (p = 0.07). Lipoprotein(a) was not associated with sCD154 (p = 0.38). sCD154 levels were lower in patients with nephrotic syndrome (p = 0.05).

Measures of disease activity, including the physician estimate of activity (p = 0.39), the SELENA SLEDAI (p = 0.56), and serologic measures, including anti-dsDNA and complement levels, were not associated with sCD154 (Table 2).

Table 1. Levels of sCD154 (pg/ml) by demographic factors, cardiovascular risk factors, and clinical manifestations (n = 183).

Variable	Level of sCD154 (pg/ml) When Variable Is		
	Present	Absent	p
Demographic			
High school education	490.1 ± 27.4	479.7 ± 81.5	0.90
Ethnicity (African American)	476.8 ± 45.2	488.7 ± 33.3	0.83
Female	480.2 ± 26.7	518 ± 92.6	0.69
Cardiovascular risk factors			
Smoking	542.9 ± 66.3	480.8 ± 27.9	0.38
Obesity	464 ± 34.0	501 ± 38.2	0.467
Diabetes mellitus	488.4 ± 104.1	490.3 ± 26.5	0.98
Hypertension	455.2 ± 37.5	521.1 ± 35.2	0.20
Cholesterol > 200 mg/dl	467.5 ± 32.6	527.2 ± 41.7	0.26
Triglycerides, mg/dl	431.4 ± 64.5	501 ± 31.8	0.33
Clinical manifestations			
Nephrotic syndrome	371.0 ± 65.7	511.3 ± 27.7	0.05
Deep venous thrombosis	354.5 ± 83	504.4 ± 26.9	0.08
Malar rash	491.1 ± 32.5	488.6 ± 42.5	0.96
Discoid lupus	477.1 ± 54.6	493.9 ± 29.2	0.78
Mouth ulcers	465.3 ± 35.3	518.2 ± 37.5	0.30
Pleurisy	501.3 ± 40.4	482.5 ± 33.5	0.72
Pericarditis	505.0 ± 55.2	486.0 ± 29.1	0.76
Arthritis	479.2 ± 29.2	528.4 ± 54.5	0.42
Leukopenia	483.8 ± 37.2	496.0 ± 35.8	0.81

Table 2. Levels of sCD154 (pg/ml) by the presence or absence of disease activity measures (n = 183).

Disease Activity Variable	Level of sCD154 When Variable Is		
	Present	Absent	p
Physician's estimate of activity ≥ 1.0	458.3 ± 34.4	502.5 ± 36.82	0.39
SLEDAI ≥ 4 (vs < 4)	457.2 ± 41.57	492 ± 31.16	0.56
Anti-dsDNA-positive	482.4 ± 33.3	501.7 ± 40.6	0.71
Low C3	481.4 ± 30.2	484.9 ± 42.1	0.09
Low C4	476.2 ± 31.3	489.1 ± 39.5	0.80

Table 3. Levels of sCD154 by the presence or absence of treatment variables (n = 183).

Treatment	Level of sCD154 When Variable Is		
	Present	Absent	p
Prednisone	471.7 ± 35.4	469.5 ± 30.6	0.96
Aspirin	497.7 ± 55.2	488.1 ± 29.1	0.87
Oral contraceptive	457.1 ± 108.4	490.4 ± 27.3	0.76
Hormone therapy	363.3 ± 57.9	519.9 ± 29.4	0.01
Immunosuppressive	427.4 ± 37.3	544.5 ± 34.7	0.02

Immunosuppressive (p = 0.02) and hormone therapy (p = 0.01) were associated with lower levels of soluble CD154 (Table 3). In a multiple logistic regression analysis, even after adjusting for immunosuppressive therapy, there was no association of sCD154 levels in subjects with coronary calcium or carotid plaque versus those without (p = 0.49 and p = 0.56).

Table 4. Levels of sCD154 in subclinical atherosclerosis (n = 183).

Variable	Level of sCD154 (pg/ml) When Variable Is Present	Absent	p
Coronary calcium	459.5 ± 39.7	500.1 ± 33.6	0.43
Carotid plaque	474 ± 29.2	526 ± 51	0.45

sCD154 was not associated with coronary calcium (p = 0.43) or with carotid plaque severity (p = 0.45) (Table 4). A trend of lower levels of sCD154 was seen in patients who had carotid plaque (474 vs 526) compared to those without carotid plaque.

DISCUSSION

The pathogenesis of atherosclerosis in SLE is multifactorial, including traditional cardiovascular risk factors, prothrombotic factors, inflammation, and immunoregulatory dysfunction[28-30].

Soluble CD154 levels have been reported to be elevated in the serum of SLE patients compared to controls[20,21,25]. Our study did not include controls, and does not negate this previous work showing an association of sCD154 with SLE. Two previous studies of 26 SLE patients each[21,25] found an association of sCD154 with anti-dsDNA[21], disease activity[21], or very high disease activity (SLEDAI > 10)[25]. Our larger sample and use of multiple measures of disease activity (physician's global assessment, SELENA SLEDAI, anti-dsDNA, complement) revealed no association of disease activity with sCD154. Subjects taking immunosuppressive medications had lower levels of sCD154. It is known that nucleoside analogs with immunostimulatory effects can increase sCD154 levels in patients with human immunodeficiency virus[31-33]. Statins have been shown to lower sCD154 levels in patients with hypercholesterolemia and coronary artery disease[34,35], but none of our patients was taking a statin.

Ours is the first study of sCD154 in SLE to utilize multiple measures of subclinical atherosclerosis. We found no association of sCD154 with carotid plaque or with coronary calcium. No association of sCD154 with carotid plaque was reported by Roman, *et al* in 197 SLE patients[22]. Our results are also consistent with the Dallas Heart Study, in which no association of sCD154 with coronary artery calcium or aortic plaque was found in the general population[17]. Indeed, in our study those with subclinical atherosclerosis actually had lower, not higher, levels of sCD154. Further, there was no association of sCD154 with traditional or novel cardiovascular risk factors in SLE.

Our study has some limitations. Atherosclerosis in SLE is multifactorial, and in a subset of lupus patients not yet defined sCD154 might play a role. Further, our study examined subclinical atherosclerosis, not actual cardiovascular events.

Our study found no relationship between sCD154 and either subclinical atherosclerosis or SLE disease activity. This suggests that sCD154 is not an important mediator of accelerated atherosclerosis or of disease activity in SLE. However, it does not rule out that sCD154 is involved in acute coronary syndromes or in unstable atherosclerotic plaque in SLE.

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