Increased Concentration of Proatherogenic Inflammatory Cytokines in Systemic Lupus Erythematosus: Relationship to Cardiovascular Risk Factors

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ABSTRACT. ***Objective.*** To examine the hypothesis that patients with systemic lupus erythematosus (SLE) have increased concentrations of interleukin-6 (IL-6), IL-8, and monocyte chemoattractant protein-1 (MCP-1) and that these cytokines are associated with coronary risk factors and atherosclerosis.

Methods. Plasma IL-6, MCP-1, and serum IL-8 (pg/ml) concentrations were measured in 74 patients with SLE and in 85 controls. Clinical characteristics, homocysteine, lipids, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and coronary artery calcification as detected by electron beam computed tomography were measured.

Results. IL-6 (13.2 ± 13.8 pg/ml vs 6.7 ± 3.2 pg/ml, $p < 0.001$) and MCP-1 (264.2 ± 581.8 pg/ml vs 131.0 ± 63.7 pg/ml, $p < 0.001$) concentrations were higher in patients with lupus than in controls. IL-8 concentrations did not differ between patients and controls ($p = 0.86$). In patients, IL-6 concentrations were correlated with CRP ($p < 0.001$), ESR ($p < 0.001$), SLE disease activity index (SLEDAI, $p = 0.003$), and body mass index (BMI, $p = 0.003$). IL-6 concentrations were inversely correlated with HDL cholesterol ($p = 0.01$). MCP-1 concentrations were correlated with SLEDAI ($p = 0.01$), ESR ($p = 0.04$), and triglycerides ($p = 0.03$). After controlling for age, sex, disease activity, SLICC damage index, smoking status, and systolic blood pressure, IL-6 was associated with coronary calcification (odds ratio, OR = 1.07, $p = 0.035$). Similar models found no association between MCP-1 or IL-8 with coronary artery calcification.

Conclusion. Patients with SLE have increased concentrations of IL-6 and MCP-1. These cytokines are associated with increased inflammation, BMI, and adverse lipid profiles. IL-6 is associated with burden of atherosclerosis in SLE. (J Rheumatol 2006;33:539–45)

Key Indexing Terms:
CYTOKINES
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Patients with systemic lupus erythematosus (SLE) have an increased risk of premature coronary and extracoronary atherosclerosis[1,2]. In young women with SLE the incidence of myocardial infarction is increased more than 50-fold compared with controls of similar age[3]. Furthermore, patients with SLE are 2.3 times more likely to be hospitalized because of myocardial infarction[3,4], and data from autopsies show that more than 50% of patients with SLE have moderate to severe atherosclerosis[5].

Atherosclerosis is an inflammatory state and there is increasing evidence that several cytokines, including interleukin 6 (IL-6), IL-8, and monocyte chemoattractant protein-1 (MCP-1) play a key role in its pathogenesis[6-8]. For example, IL-6 is important in the recruitment of inflammatory cells and in lipid homeostasis and its concentrations are increased in patients with unstable angina and are associated with prognosis[9-11]. MCP-1 plays a role in the early inflammatory cellular response and is associated with atherosclerosis in animal models and in the general population[8,12-15]. IL-8 has been linked to monocyte recruitment, and macrophages from atherosclerotic plaques show an enhanced capacity to produce IL-8, thought to result in the generation of unstable plaque. Moreover, IL-8 induces the migration of neutrophils and vascular endothelial cells and stimulates migration and proliferation of vascular smooth muscle cells, and increased concentrations of IL-8 were found in infarct-related coronary artery samples[16-20].

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SLE is a disease characterized by activated T cells, circulating autoantibodies, and elevated concentrations of proinflammatory cytokines. Several cytokines such as interferon gamma (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-4 have a role in animal models of atherosclerosis and have been implicated in studies in humans[21-23]. In addition, IL-6 exacerbates glomerulonephritis in animal models of lupus, and urinary and serum concentrations are increased in patients with SLE[24-25]. Evidence regarding the role of IL-8 in SLE is controversial. Initially IL-8 was associated with lupus activity, and monoclonal anti-dsDNA antibody was shown to enhance the expression and release of IL-8 and other cytokines. However, other studies found no association between urinary IL-8 concentrations and exacerbation of renal disease, and plasma IL-8 concentrations of patients with neuropsychiatric lupus did not differ from those of controls[26-29].

The role of proinflammatory proatherogenic cytokines in the pathogenesis of accelerated atherosclerosis in lupus has not been established. Therefore, we examined the hypothesis that patients with SLE have increased concentrations of IL-6, IL-8, and MCP-1 and that these are associated with coronary risk factors including coronary artery atherosclerosis.

MATERIALS AND METHODS

Patients. We studied 74 patients with lupus and 85 controls who were frequency-matched for age, race, and sex. Data regarding the frequency of coronary artery calcification in this ongoing study have been reported[1]. Consecutive eligible patients older than 18 years who met the classification criteria for SLE[30] and had duration of disease longer than one year were enrolled. Controls did not meet classification criteria for lupus or any other rheumatic disease. All subjects with a history of cardiovascular disease (previous stroke, myocardial infarction, and/or angina) were excluded. Patients were recruited from local rheumatologists, through a Lupus Foundation newsletter, and by advertisements. Controls were recruited from the patients' acquaintances, by advertisement, and from a database of volunteers maintained by the General Clinical Research Center. The study was approved by the Institutional Review Board of Vanderbilt University Hospital and all subjects gave written informed consent.

Clinical assessment. Information was obtained through a structured interview, physical examination, and in patients, by review of medical records. Blood pressure was determined as the average of 2 measurements obtained 5 minutes apart after subjects had rested quietly in the supine position for at least 10 minutes. Height (m) and weight (kg) were measured and body mass index (BMI) calculated. Disease activity was measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[31], and cumulative organ damage was calculated based on the Systemic Lupus International Collaborating Clinics (SLICC) damage index[32].

Cytokine measurements. Plasma and serum were separated by centrifugation and stored at –70°C. IL-6 and MCP-1 (pg/ml) plasma concentrations and IL-8 serum concentrations were determined by enzyme linked immunosorbent assay (ELISA) using commercial kits (R&D system, Minneapolis, MN, USA).

Other laboratory tests. Blood was collected while subjects were fasting for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, lipoprotein(a) [Lp(a)], and homocysteine concentrations. In patients with lupus, C-reactive protein (CRP), Westergren erythrocyte sedimentation rate (ESR), and total hemolytic complement (CH50) were also measured.

Coronary artery calcification. All subjects underwent imaging with an Imatron C-150 scanner (Imatron, South San Francisco, CA), as described[1]. This is a noninvasive imaging technique to detect coronary artery calcification as a measure of coronary atherosclerosis burden. The extent of coronary calcification demonstrates a moderate correlation with coronary artery luminal stenosis as detected by angiography and a strong correlation with atherosclerosis in pathologic specimens. Furthermore, it is predictive of future coronary events[33-35]. Coronary artery calcification was calculated as described by Agatston, *et al* [36], and the sum of the scores for all arterial lesions provided an overall score for each subject.

Statistical methods. Demographic characteristics are presented as means and standard deviations for continuous variables and as frequencies and percentages for categorical variables. The differences among cases and controls were determined by Wilcoxon rank sum tests or Pearson's chi square test, as appropriate.

IL-6, IL-8, and MCP-1 concentrations were compared in patients with lupus and controls using Wilcoxon rank sum tests. Spearman correlations between IL-6, IL-8, and MCP-1 concentrations with other continuous variables were calculated.

Adjusted odds ratios (OR) were obtained with the use of multiple logistic regression models to determine independent associations between the presence or absence of any coronary artery calcium and concentrations of cytokine after controlling for pre-determined confounders including age, sex, pack-years history of smoking, systolic blood pressure, disease activity, and disease severity. No corrections for multiple comparisons were performed. All analyses used a 2-sided significance level of 5% and were performed with STATA 8.2.

RESULTS

Demographic characteristics and cardiovascular risk factors are shown in Table 1. Patients with lupus (n = 74) and controls (n = 85) were of similar age (40.9 ± 12.3 yrs and 41.5 ± 11.2 yrs, respectively) and sex (90.5% and 90.6% female), and had similar systolic blood pressure (120.8 ± 19.2 mmHg vs 120.0 ± 15.5 mmHg) and BMI (27.8 ± 6.2 kg/m^2 vs 26.8 ± 5.2 kg/m^2). The differences were not statistically significant, but patients with SLE tended to have higher diastolic blood pressure (75.9 ± 14.3 mmHg vs 71.4 ± 9.9 mmHg) and more cumulative pack-years of smoking (6.1 ± 11.0 yrs vs 4.4 ± 12.7 yrs). As previously described, the lipid profile differed between patients and control subjects[1]. LDL concentrations were significantly higher in controls than in patients with lupus (111.7 ± 34.3 mg/dl vs 101.5 ± 36.4 mg/dl, p = 0.03 mg/dl); but triglyceride concentrations were higher in patients with lupus than in controls (118.9 ± 56.2 mg/dl vs 99.8 ± 54.2 mg/dl, p = 0.02). Concentrations of total cholesterol, HDL and Lp(a) lipoproteins were similar in both groups.

Patients with lupus had mean duration of disease of 9.6 ± 8.2 years, mean SLEDAI score of 3.6 ± 3.5, mean SLICC damage index of 1.0 ± 1.3, CH50 of 202.5 ± 67.0 units, ESR of 23.8 ± 22.7 mm/h and CRP of 7.3 ± 9.2 mg/l. Patients with lupus had higher homocysteine concentrations (9.5 ± 3.6 µmol/l vs 8.3 ± 6.6 µmol/l, p < 0.001) and higher coronary calcification scores (68.0 ± 239.2 vs 7.1 ± 37.3 Agatston units, p = 0.002) than controls.

Cytokine concentrations. Figure 1 shows the cytokine concentrations among patients and controls. IL-6 (13.2 ± 13.8 pg/ml vs 6.7 ± 3.2 pg/ml, p < 0.001) and MCP-1 (264.2 ± 581.8 pg/ml vs 131.0 ± 63.7 pg/ml, p < 0.001) concentrations

Table 1. Characteristics (means ± standard deviations) of patients with SLE and controls. Significance values were determined using the Wilcoxon rank sum test or Pearson's chi square test as appropriate.

Variables	Patients (n = 74)	Control subjects (n = 85)	p
General characteristics			
Age, yrs	40.9 ± 12.3	41.5 ± 11.2	0.62
Males, %	7 (9.5)	8 (9.4)	0.99
Systolic blood pressure, mmHg	120.8 ± 19.2	120 ± 15.5	0.77
Diastolic blood pressure, mmHg	75.9 ± 14.3	71.4 ± 9.9	0.07
BMI, kg/m^2	27.8 ± 6.2	26.8 ± 5.2	0.39
Cumulative smoking, pack-yrs	6.1 ± 11.0	4.4 ± 12.7	0.06
Disease characteristics			
Duration of disease, yrs	9.6 ± 8.2	NA	NA
SLEDAI	3.6 ± 3.5	NA	NA
SLICC	1.0 ± 1.3	NA	NA
CH50, units	202.5 ± 67.0	NA	NA
ESR, mm/h	23.8 ± 22.7	NA	NA
CRP, mg/l	7.3 ± 9.2	NA	NA
Lipid profile, mg/dl			
Total cholesterol	172.2 ± 46.6	181.0 ± 40.6	0.08
High-density lipoprotein	47.0 ± 16.0	49.3 ± 15.7	0.37
Low-density lipoprotein	101.5 ± 36.4	111.7 ± 34.3	0.03
Triglycerides	118.9 ± 56.2	99.8 ± 54.2	0.02
Lipoprotein (a)	26.7 ± 30.7	26.4 ± 34.6	0.84
Other laboratory results			
Hemoglobin, g/dl	12.9 ± 1.7	13.5 ± 3.5	0.61
Creatinine, mg/dl	0.9 ± 0.3	0.8 ± 0.2	0.87
Albumin, mg/dl	3.7 ± 0.5	3.7 ± 0.5	0.78
Homocysteine, µmol/l	9.5 ± 3.6	8.3 ± 6.6	< 0.001
Coronary calcification (Agatston score)	68.0 ± 239.2	7.1 ± 37.3	0.002

Missing data: Patients with SLE: CH50 (n = 1); control subjects: Homocysteine (n = 3). BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

were significantly higher in patients with lupus than controls. IL-8 concentrations did not differ significantly among patients and controls (18.2 ± 38.5 pg/ml vs 11.6 ± 14.6 pg/ml, p = 0.86).

Relationship between cytokine concentrations and coronary risk factors in patients with lupus. Among patients with lupus, IL-6 concentrations were significantly correlated with BMI (rho = 0.34, p = 0.003), disease activity as determined by the SLEDAI score (rho = 0.34, p = 0.003), and other inflammatory markers: ESR (rho = 0.42, p < 0.001) and CRP (rho = 0.50, p < 0.001), MCP-1 (rho = 0.41, p < 0.001) and IL-8 (rho = 0.26, p = 0.02). IL-6 was also inversely correlated with HDL cholesterol concentration (rho = –0.29, p = 0.01) but not with LDL cholesterol.

MCP-1 concentrations were correlated with the SLEDAI score (rho = 0.29, p = 0.01), ESR (rho = 0.24, p = 0.04), and triglycerides (rho = 0.25, p = 0.03). IL-8 was negatively correlated with HDL (rho = –0.28, p = 0.02). IL-6 (p = 0.13), MCP-1 (p = 0.61), and IL-8 concentrations (p = 0.61) were not associated with coronary artery calcification in a univariate analysis (Table 2).

Older age (p < 0.001) and male sex (p = 0.01) were more common, and BMI (p = 0.04), creatinine concentrations (p = 0.004), and Lp(a) (p = 0.03) were higher in patients with coronary artery calcification than in patients without coronary calcification. There was a trend toward a higher concentration of IL-6 among patients with coronary calcification; however, cytokine concentrations did not differ significantly between patients with and without coronary artery calcification (IL-6: p = 0.11, IL-8: p = 0.82, and MCP-1: p = 0.41).

Calcification was present in 20 of 74 patients. Logistic regression models were used to obtain adjusted OR for the presence of coronary calcium. After controlling for age, sex, disease activity, cumulative damage as determined by the SLICC damage index, smoking status, and systolic blood pressure, the adjusted OR for the presence of coronary artery calcification with an increase of 1 pg/ml of IL-6 was significant (OR = 1.07, p = 0.035). Similar models found no association between MCP-1 (OR = 1.0, p = 0.70) and IL-8 (OR = 0.98, p = 0.39) with coronary artery calcification. This association remained significant after further adjustment for ESR (OR = 1.07, p = 0.04) and was of borderline significance after further adjustment for CRP (OR = 1.07, p = 0.06). IL-6 and CRP are not biologically independent; IL-6 is the major determinant of most acute phase proteins, including CRP[37,38].

DISCUSSION

This study extends previous observations that atherosclerosis

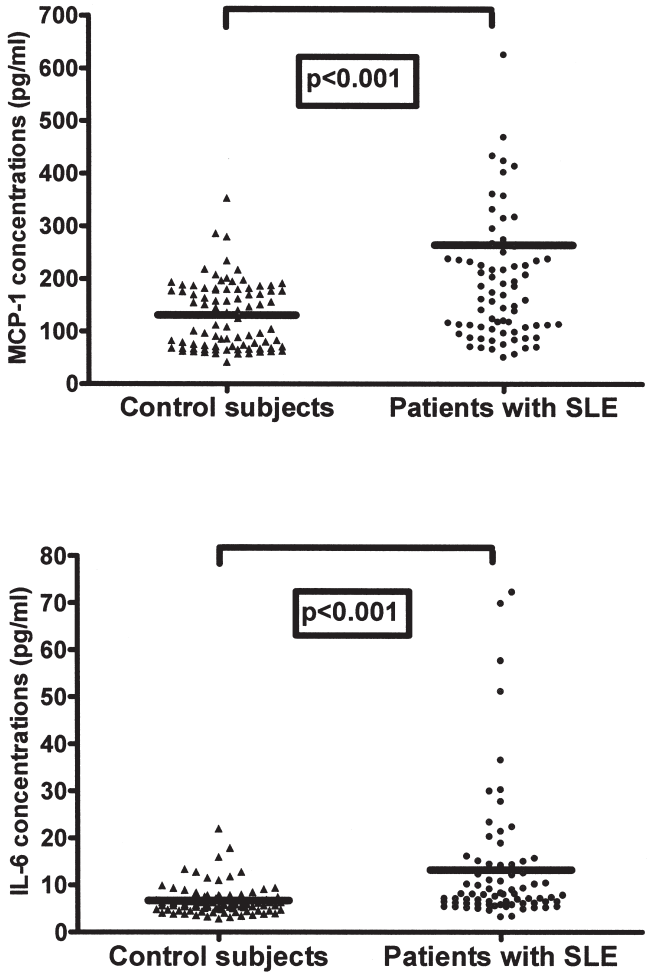

Figure 1. Horizontal lines represent mean concentrations of MCP-1 and IL-6. Wilcoxon rank sum test was used to determine p values for differences in concentration between patients and controls.

is increased in patients with lupus[1,2] by exploring potential relationships between atherosclerosis and proinflammatory cytokines in patients with SLE. Our results show that patients with SLE have higher concentrations of 2 proatherogenic cytokines (IL-6 and MCP-1) and suggest a link between IL-6 and proinflammatory markers, disease activity, BMI, and lower levels of HDL. This study also shows that MCP-1 may be associated with disease activity and triglyceride concentrations in patients with lupus.

Animal studies suggest a fundamental role for IL-6 and MCP-1 in the pathogenesis of atherosclerosis. Thus, MCP-1 concentrations correlated with monocyte infiltration in atherosclerotic lesions and anti-MCP-1 gene therapy limited progression and destabilization of atherosclerosis in animal models[13,39]. In addition, IL-6 enhanced fatty lesion and atherosclerotic plaque formation in susceptible mice[10,40]. IL-8 is a chemokine present in atherosclerotic lesions. Animal studies show that mice deficient in the receptor for IL-8, CXCR2, had significantly reduced progression of atherosclerosis. IL-8 also recruits macrophages and may contribute to plaque formation[16,41,42]. Data regarding IL-8 in SLE are limited and our results suggest that concentrations of IL-8 are not significantly elevated nor are they associated with atherosclerosis in patients with lupus.

Epidemiological studies also support the role of these cytokines in the pathogenesis of coronary atherosclerosis in humans. Basal IL-6 concentrations were higher among subjects who subsequently developed myocardial infarction compared with healthy controls[43]. Furthermore, IL-6 concentrations were elevated in patients with unstable angina and correlated with CRP concentrations. A population-based study showed that individuals with the highest quartile of MCP-1 concentrations had a 2-fold increased risk of prevalent coronary artery calcification compared to the lowest quartile[14]. Also, elevated basal concentrations of MCP-1 were linked to traditional risk factors and to a 50% increase in the risk of myocardial infarction or mortality in patients with acute coronary syndrome[15].

Traditional cardiovascular risk factors such as age, blood pressure, and higher concentrations of total and LDL cholesterol, lupus-related factors such as duration of disease and higher disease activity and damage scores, and markers of inflammation such as CRP and cytokine concentrations have been suggested to play a role in the accelerated atherosclerosis found in patients with lupus[2,44-47]. Thus, the fact that elevated IL-6 and MCP-1 concentrations are also associated with several of these traditional and disease-related risk factors further supports the notion that these cytokines may play a role in the mechanisms underlying increased atherosclerosis in patients with lupus.

The lack of a significant association between IL-6 and MCP-1 concentrations and coronary artery calcification in the univariate analysis and the weak association of IL-6 after controlling for potentially confounding covariates may not reflect the role that these 2 cytokines may play in the pathogenesis of atherosclerosis in patients with lupus. Thus, IL-6 and MCP-1 concentrations could be important early in the pathogenesis of atherosclerotic process, before the lesion is established and therefore before the lesion is calcified[40]. Also, although coronary calcification provides an excellent measure of the atherosclerosis burden and has been predictive of cardiovascular events in virtually every study[48-50], it does not specifically identify high risk (unstable) plaques. Inflammatory cytokines, in addition to participating in the pathogenesis of atherosclerosis, could play a role in destabilizing the fibrous cap and thus facilitate plaque rupture, the initial event in coronary thrombosis[51].

The association between inflammation and atherosclerosis in patients with SLE has been explored with other markers. Recently CRP was associated with an increase risk of occurrence of cardiovascular arterial events, and African-American and Hispanic patients with SLE carrying the CRP GT 20 variant were more likely to develop vascular arterial events[52,53].

Table 2. Relationship between plasma concentrations of IL-6, MCP-1, IL-8 and characteristics. Significance values were calculated using Spearman's correlations.

	IL-6		IL-8		MCP-1	
	Rho	p	Rho	p	Rho	p
General characteristics						
Age, yrs	0.06	0.59	–0.06	0.62	0.08	0.52
Systolic blood pressure, mmHg	0.05	0.11	–0.02	0.88	0.08	0.48
Diastolic blood pressure, mmHg	0.11	0.36	–0.01	0.96	–0.01	0.94
BMI, kg/m^2	0.34	0.003	–0.08	0.51	0.14	0.22
Cumulative smoking, pack-yrs	0.01	0.96	0.03	0.82	–0.04	0.75
Disease characteristics						
Duration of disease, yrs	0.03	0.79	0.03	0.78	0.14	0.24
SLEDAI	0.34	0.003	0.15	0.22	0.29	0.01
SLICC	0.00	0.99	–0.14	0.23	0.07	0.55
CH50, units	–0.07	0.57	–0.19	0.10	0.18	0.11
Laboratory results						
Hemoglobin, g/dl	–0.07	0.55	–0.04	0.74	–0.16	0.18
Creatinine, mg/dl	0.02	0.86	0.04	0.73	0.14	0.25
Albumin, mg/dl	–0.23	0.05	0.11	0.35	–0.25	0.03
Homocysteine, µmol/l	0.21	0.08	0.22	0.06	0.08	0.50
ESR, mm/h	0.42	< 0.001	0.20	0.09	0.24	0.04
CRP, mg/l	0.50	< 0.001	0.04	0.73	0.19	0.11
Lipid profile, mg/dl						
Cholesterol	–0.01	0.93	–0.14	0.23	0.09	0.47
High-density lipoprotein	–0.29	0.01	–0.28	0.02	–0.08	0.51
Low-density lipoprotein	0.05	0.69	–0.10	0.39	0.09	0.43
Triglycerides	0.22	0.06	0.08	0.50	0.25	0.03
Lipoprotein (a)	0.16	0.17	0.10	0.39	0.21	0.08
Inflammatory cytokines, pg/ml						
MCP-1	0.41	< 0.001	0.20	0.08	1.0	—
IL-8	0.26	0.02	1.0	—	0.20	0.08
IL-6	1.00	—	0.26	0.02	0.41	< 0.001
Coronary calcification (Agatston units)	0.18	0.13	–0.03	0.80	0.06	0.61

In contrast to other inflammatory conditions, such as rheumatoid arthritis, in SLE CRP does not correlate well with IL-6, suggesting that the regulatory mechanisms of these markers of inflammation may be different in this disease[54-56].

One of the limitations of our study is that a cross-sectional design has a limited capacity to establish a causal relationship because it does not address the temporal relationship between exposure and outcomes. Longitudinal studies would be of interest.

In conclusion, our results show that patients with SLE have increased concentrations of IL-6 and MCP-1 and that these cytokines correlate with some traditional coronary risk factors. Furthermore, IL-6 showed a modest association with burden of coronary atherosclerosis. The fact that IL-6 was associated with coronary artery atherosclerosis even after adjusting for ESR suggests that it may provide information additional to that provided by markers of inflammation such as CRP and ESR. Currently, IL-6 and MCP-1 are promising targets to treat lupus, and studies to investigate whether these interventions will control the accelerated atherosclerotic process of these patients will be of interest[57,58].

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