Genetically Determined Serum Levels of Mannose-Binding Lectin Correlate Negatively with Common Carotid Intima-Media Thickness in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Patients with systemic lupus erythematosus (SLE) have excess cardiovascular morbidity and mortality due to accelerated atherosclerosis that cannot be attributed to traditional cardiovascular risk factors alone. Variant alleles of the mannose-binding lectin gene (*MBL2*) causing low serum concentrations of functional mannose-binding lectin (MBL) are associated with SLE and development of atherosclerosis. Recent studies show that these variant alleles are associated with increased risk of arterial thrombosis and cardiovascular disease in patients with SLE. Intima-media thickness of the common carotid artery (ccIMT) is a validated noninvasive anatomic measure of subclinical atherosclerosis. In a cross-sectional study we examined the relation among ccIMT, *MBL2* genotypes, and serum concentrations of MBL.

Methods. The *MBL2* extended genotypes (YA/YA, YA/XA, XA/XA, YA/YO, XA/YO, YO/YO) and serum concentrations of MBL were determined in 41 outpatients with SLE. ccIMT was measured by means of ultrasonography. Traditional and nontraditional cardiovascular risk modifiers were assessed and controlled for.

Results. Using nonparametric Mann-Whitney tests we found a significant difference in ccIMT between low-expressing (XA/XA+YA/YO+XA/YO+YO/YO) and high-expressing (YA/YA+YA/XA) *MBL2* genotypes (p = 0.034). The difference in ccIMT remained significant in multivariable analysis adjusting for traditional and nontraditional cardiovascular risk modifiers (p = 0.049). ccIMT was negatively correlated to serum concentrations of MBL (Spearman rho = –0.33, p = 0.037). This relation also remained significant in multivariable analysis (p = 0.042).

Conclusion. In this group of SLE patients, *MBL2* low-expressing genotypes and low serum levels of MBL were correlated with ccIMT, independent of the effects of traditional and nontraditional cardiovascular risk modifiers. (First Release July 1 2010; J Rheumatol 2010;37:1815–21; doi:10.3899/jrheum.100158)

Key Indexing Terms:
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Patients with systemic lupus erythematosus (SLE) have increased incidence of preclinical atherosclerosis[1,2,3,4] and cardiovascular disease (CVD) compared to the general population[5,6]. The increased cardiovascular risk in SLE cannot be fully explained by traditional cardiac risk factors alone[7,8]. Accumulating evidence indicates that inflammation plays a key role in development of atherosclerosis from plaque initiation to plaque rupture and thrombus formation[9,10]. SLE is a chronic inflammatory disease with increased levels of proatherogenic mediators[11,12,13]. Several studies suggest that disease-specific factors other than traditional risk factors may play an additional role in development of atherosclerosis and CVD in SLE[8,14]. Potent antiinflammatory medication provides survival benefits by reducing development of atherosclerosis and risk of coronary disease in

patients with SLE[14,15]. Thus, it is likely that factors modulating the inflammatory response in SLE may affect development of atherosclerosis and CVD in SLE patients.

Mannose-binding lectin (MBL) is a liver-derived serum protein involved in innate immune defense[16]. It binds microorganisms and cellular debris through the carbohydrate recognition domain. Serum MBL can directly opsonize pathogens and enhance the activity of phagocytic cells or it can activate complement via the lectin pathway. Differences in MBL serum concentrations can partly be explained by structural variant alleles in the human MBL gene (*MBL2*) on chromosome 10[17]. The normal genotype is associated with the highest serum concentrations of MBL. Heterozygosity for *MBL2* structural variant alleles causes an average 85%–90% drop in the serum concentration of functional MBL and homozygosity for structural variant alleles are devoid of functional MBL. The normal *MBL2* allele is denoted A, and the common designation for the variant alleles is O. In addition to the effect of the structural allelic variants, polymorphisms in the promoter region of *MBL2* gene affect serum levels of MBL. In particular, a base substitution at codon –221 (G to C; promoter allele X) is associated with lower serum concentrations of MBL.

Studies indicate an association between MBL deficiency and susceptibility for SLE[18], and low MBL concentrations have been related to the clinical course of SLE, e.g., complicating infections[19] and renal involvement[20]. In a followup study we observed that homozygosity for *MBL2* variant alleles was associated with an increased risk of arterial thrombosis among patients with SLE[21]. This is in accord with another study where SLE patients with MBL deficiency had a 3.1 increased risk of CVD[22]. Studies of various populations have shown that the presence of dysfunctional alleles and low serum levels of MBL are associated with increased risk of atherosclerosis and coronary artery disease[23,24,25,26].

Intima-media thickness of the common carotid artery (ccIMT) determined by high-resolution B-mode ultrasonography is a useful noninvasive anatomic measure of subclinical CVD and constitutes an excellent surrogate marker of generalized atherosclerosis and coronary artery disease[27]. Increased ccIMT has been observed in patients with SLE compared to controls[1,4] and correlates with several traditional and nontraditional cardiovascular risk factors in SLE[1,4,28,29].

Based on these findings we hypothesized that low-expressing *MBL2* genotypes and low serum levels of MBL are associated with increased subclinical atherosclerosis measured by ccIMT in patients with SLE.

MATERIALS AND METHODS

Patients. Forty-one consecutive unrelated white Danish outpatients with SLE attending the Department of Rheumatology, Rigshospitalet, Copenhagen University Hospital, were included from August 2005 to July 2006. All patients fulfilled the American College of Rheumatology (ACR) classification criteria for SLE[30]. At inclusion, all patients were subjected to a detailed interview and underwent a clinical examination. Fasting serum samples were collected and stored at –80°C. Within 24 weeks of recruitment ccIMT was measured in all patients. The study was approved by the local scientific ethical committee (KF 01 261669). Written informed consent was obtained from each patient.

Clinical and laboratory assessment. Traditional cardiovascular risk modifiers included in this study were: sex, age, blood pressure calculated as mean blood pressure (1/3 × systolic blood pressure + 2/3 × diastolic blood pressure), smoking calculated as "pack-years" [mean number of packs (= 20 cigarettes) per day × number of years smoking], body mass index (BMI), total cholesterol, homeostatic model assessment of insulin resistance (HOMA1-IR) [(insulin (mU/l) × glucose (mmol/l))/22.5][31]. Nontraditional cardiovascular risk modifiers were disease duration, highly sensitive C-reactive protein (hsCRP), SLE Disease Activity Index (SLEDAI)[32], Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI)[33], and treatment-ever with glucocorticoids or other immunosuppressants. All patients were interviewed and examined by the same physician. Clinical charts were used to verify information regarding treatment with antiinflammatory drugs.

Genotyping and detection of MBL serum concentration. Detection of *MBL2* alleles was as described[34]. All patients were genotyped for the three *MBL2* structural polymorphisms and for the promoter polymorphism in position —221 (X/X, X/Y, Y/Y). The following *MBL2* extended genotypes were tested for: YA/YA, YA/XA, XA/XA, YA/YO, XA/YO, and YO/YO. Serum concentrations of MBL were measured in a double ELISA based on a monoclonal anti-MBL antibody[35]. Low-expressing genotypes were defined to include XA/XA, YA/YO, XA/YO, and YO/YO based on the genotypes expressing the lowest MBL serum concentrations (Figure 1), while YA/YA and YA/XA were defined as high-expressing genotypes due to their associations with the highest MBL serum concentrations.

ccIMT measurement. Measurement of ccIMT was performed by using an Acuson Cypress ultrasound apparatus with a 7 MHz linear probe. A still picture of the common carotid artery proximal to the bulbus caroticus was produced, ensuring a distinct picture of intima and media on the far wall of the vessel (the intima-media). Thickness was measured 3 times using the

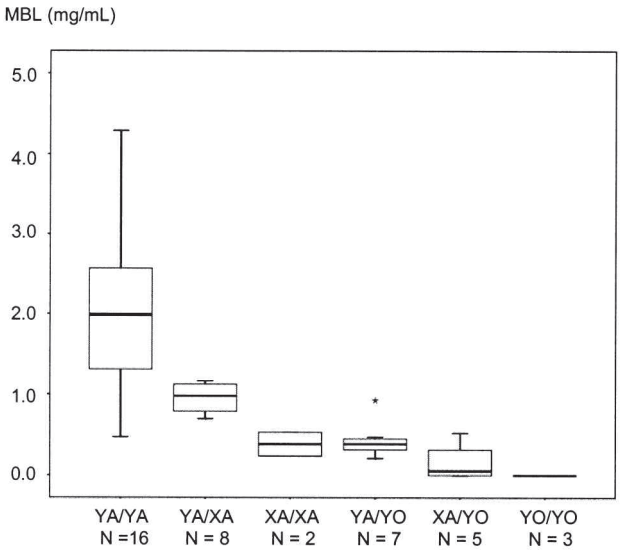

Figure 1. Serum concentrations of mannose-binding lectin (MBL) in relation to *MBL2* extended genotypes in 41 patients with SLE. Data are presented as box plots: boxes represent 25th to 75th percentiles, lines within boxes the median, and the lines outside represent 10th and 90th percentiles. *Outliers.

electronic calipers of the ultrasound apparatus and the picture was digitally stored for future reference. Measurements were performed on both carotid arteries. A mean for both arteries was calculated and used in analysis. To ensure correct calibration of ccIMT measurements these were also performed in 10 healthy subjects (8 women, 2 men) with a median age of 44 years (range 25–72 yrs).

Statistical analysis. The differences in ccIMT between SLE patients and healthy individuals and between low-expressing and high-expressing *MBL2* genotypes were examined by nonparametric Mann-Whitney test. Spearman's rank correlation analysis was performed to examine the relation between ccIMT and MBL serum concentrations. Multivariable regression analyses were performed to examine the relationship among ccIMT, *MBL2* low- and high-expressing genotypes, serum MBL, traditional and nontraditional risk factor variables. Due to the number of study subjects, the number of variables included into the regression model had to be limited, focusing on MBL, demographics, traditional cardiovascular risk factors, disease activity, and medication. To fit the linear regression model regarding linearity, variance homogeneity, and normal distribution, ccIMT was log-transformed. Processing of study data was done using SPSS 15. Statistical significance was defined as p value < 0.05.

RESULTS

Clinical and laboratory variables for all patients at inclusion are shown in Table 1.

The median ccIMT of SLE patients was 0.57 mm (range 0.37 to 0.98 mm), which was significantly thicker (p = 0.029) than in the healthy subjects, where median ccIMT was 0.46 mm (range 0.40 to 0.78 mm).

Median serum concentration of MBL was 0.85 mg/l (range 0 to 4.3 mg/l). Serum MBL varied with the extended *MBL2* genotypes (YA/YA, YA/XA, XA/XA, YA/YO, XA/YO, and YO/YO) as shown in Figure 1. Seventeen patients had the low-expressing genotypes (XA/XA, YA/YO, XA/YO, and YO/YO) and 24 patients had the high-expressing genotypes (YA/YA, YA/XA). Variation in ccIMT according to low-expressing and high-expressing *MBL2* genotype is shown in Figure 2. Using nonparametric Mann-Whitney test we found a significant difference in ccIMT between the low- and high-expressing *MBL2* genotypes (p = 0.034). The difference in ccIMT remained significant in multivariable analysis adjusting for traditional and nontraditional cardiovascular risk modifiers (p = 0.049; Table 2).

The relationship between ccIMT and serum concentrations of MBL is shown in Figure 3. ccIMT and serum MBL correlated negatively with a Spearman rho = –0.33 (p = 0.037). In a multivariate regression analysis we found that this negative association remained significant after adjustment for traditional and nontraditional cardiovascular risk modifiers (p = 0.042; Table 3).

Table 1. Clinical and laboratory variables assessed in the cohort of 41 patients with systemic lupus erythematosus. Except where indicated otherwise, values are median [quartiles] (range).

Sex, no. (%)	
Female	36 (88)
Male	5 (12)
Age, yrs	55 [34–54] (27–78)
Mean blood pressure, mm Hg	93 [87–105] (65–158)
Smoking (pack-years)	1.8 [0–21] (0–55)
Body mass index, kg/m^2	24 [21–26] (18–39)
Total cholesterol, mmol/l	4.5 [4.2–5.3] (3–6.4)
HOMA1-IR	1.1 [0.8–3.2] (0.3–27)
hsCRP, mg/l	2.5 [0.8–5.7] (0.3–32)
Disease duration, yrs	11 [7–17] (1–33)
SLEDAI	0 [0–2] (0–11)
SDI	1 [1–2] (0–4)
Treatment with glucocorticoids (ever), no. (%)	37 (90)
Treatment with other immunosuppressants (ever), no. (%)	37 (90)

HOMA1-IR: homeostatic model assessment of insulin resistance; hsCRP: highly sensitive C-reactive protein; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

DISCUSSION

Two studies have related MBL deficiency with CVD in SLE patients[21,22]. Since patients with SLE have increased incidence of atherosclerosis that cannot be explained solely by excess of traditional cardiovascular risk factors and since MBL deficiency has been associated with atherosclerosis and CVD in other populations[23,24,25,26], we wanted to examine if *MBL2* variant alleles and low serum MBL concentrations in SLE patients were associated with increased subclinical atherosclerosis measured by ultrasonography. Our results showed that ccIMT was increased in SLE patients compared to healthy individuals and that this increase was related to genetically determined low serum levels of MBL independently of the effects of traditional and nontraditional cardiovascular risk modifiers.

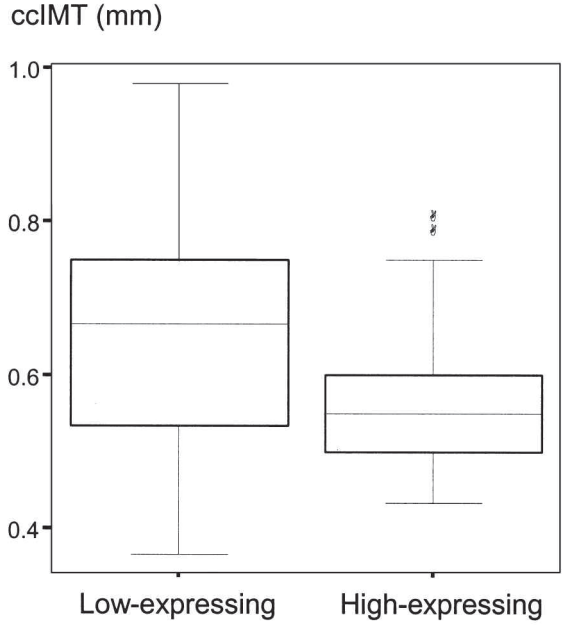

Figure 2. Common carotid intima-media thickness (ccIMT) in relation to *MBL2* low-expressing (17 patients) and high-expressing (24 patients) genotypes in 41 patients with SLE. Data are presented as box plots: boxes represent 25th to 75th percentiles, lines within boxes the median, and the lines outside represent 10th and 90th percentiles. Circles indicate outliers.

Table 2. Results of multivariable regression analysis between common carotid intima-media thickness (ccIMT)*, *MBL2* low-expressing genotypes, and traditional and nontraditional cardiovascular risk modifiers.

Factor	Regression Coefficient	95% CI	p (2-tailed)
MBL2 low-expressing genotypes	–0.076	–0.151 to –0.001	0.049
Male sex	–0.065	–0.169 to 0.039	0.209
Age	0.002	–0.001 to 0.005	0.175
Mean blood pressure	0.001	–0.001 to 0.004	0.213
Smoking (pack-years)	0.000	–0.002 to 0.003	0.786
Body mass index	0.003	–0.005 to 0.011	0.448
Total cholesterol	–0.029	–0.077 to 0.019	0.231
HOMA1-IR	–0.005	–0.013 to 0.004	0.277
hsCRP	–0.001	–0.007 to 0.005	0.837
SLEDAI	–0.002	–0.015 to 0.012	0.786
Glucocorticoids (ever)	0.040	–0.119 to 0.199	0.609
Other immunosuppressants (ever)	–0.046	–0.202 to 0.111	0.554

* To fit the model regarding linearity, variance homogeneity, and normal distribution, ccIMT was logarithmically transformed. HOMA1-IR: homeostatic model assessment of insulin resistance; hsCRP: highly sensitive C-reactive protein; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

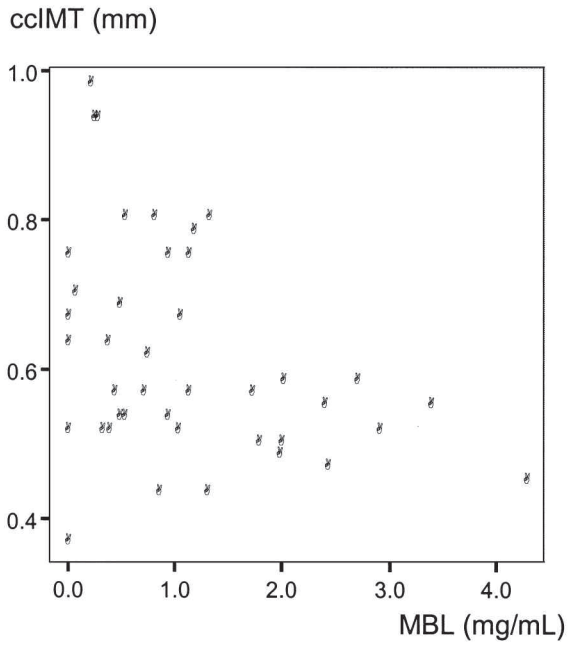

Figure 3. Common carotid intima-media thickness (ccIMT) in relation to serum concentrations of mannose-binding lectin (MBL).

ccIMT ≥ 0.60 mm is a marker of generalized atherosclerosis[36]. In our study, the mean ccIMT was 0.62 mm, in accord with previous findings in SLE patients[1,4]. Several associations between increased ccIMT and traditional cardiovascular risk factors have been observed in patients with SLE[1,4,28,29]. In some studies disease duration[29], SLEDAI[37], and SDI[38] have been associated with increased subclinical atherosclerosis in SLE patients, whereas other studies failed

Table 3. Results of multivariable regression analysis between common carotid intima-media thickness (ccIMT)*, serum MBL, and traditional and nontraditional cardiovascular risk modifiers.

Factor	Regression Coefficient	95% CI	p (2-tailed)
Serum MBL	–0.041	–0.099 to –0.008	0.042
Male sex	-0.057	–0.158 to 0.044	0.261
Age	0.001	–0.002 to 0.004	0.482
Mean blood pressure	0.001	–0.001 to 0.003	0.346
Smoking (pack-years)	0.000	–0.002 to 0.002	0.994
Body mass index	0.005	–0.004 to 0.014	0.235
Total cholesterol	–0.029	–0.076 to 0.019	0.228
HOMA1-IR	–0.006	–0.014 to 0.003	0.198
hsCRP	0.000	–0.006 to 0.006	0.972
SLEDAI	–0.002	–0.015 to 0.012	0.822
Glucocorticoids (ever)	0.034	–0.123 to 0.191	0.658
Other immunosuppressants (ever)	–0.068	–0.224 to 0.087	0.376

* To fit model regarding linearity, variance homogeneity, and normal distribution, ccIMT was logarithmically transformed. HOMA1-IR: homeostatic model assessment of insulin resistance; hsCRP: highly sensitive C-reactive protein; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

to find significant associations among disease duration, SLEDAI, SDI, and atherosclerosis in univariate and/or multivariate analysis[28,39]. Increase of ccIMT in SLE patients has also been shown to be associated with complement activation[29] and proinflammatory high-density lipoprotein[28]. Moreover, treatment with antiinflammatory drugs (in particular prednisolone) was also associated with development of atherosclerosis in patients with SLE[28,39]. Due to the relatively low number of patients included in this study only a limited number of variables could be added into the multivariable analysis. Since a higher number of studies find significant associations among traditional cardiovascular risk factors, treatment with antiinflammatory drugs, and atherosclerosis in SLE patients, we chose to focus on these risk factor variables in our analysis. *A priori* we chose not to include SDI in the analysis since it is partly defined by atherosclerotic events, and therefore per definition could be expected to covary with ccIMT. Except for age, ccIMT was unrelated to other traditional and nontraditional cardiovascular risk factors in univariate (data not shown) and multivariate analyses in our study. It is likely that differences in study design, in risk factor variables, and in medication, age and duration of disease may influence the associations studied. The discrepancies in our results could also be due to uneven distributions of confounders not accounted for, which may include MBL.

MBL can enhance the clearance of pathogens, dying host cells, and cellular debris[16], as well as circulating immune complexes[40,41,42]. Based on these capabilities several pathophysiological mechanisms may be suggested to explain the relation between deficiency of functional MBL and development of atherosclerosis in patients with SLE. A theory of

infection in atherosclerosis has not been proven, but several studies have shown that chronic infections including periodontitis and infections with *Helicobacter pylori* and *Chlamydia pneumoniae* may be associated with development of atherosclerosis and cardiovascular disease[43]. As MBL deficiency is associated with increased risk of infections in SLE patients[19]; as infection with *C. pneumoniae* leads mainly to development and progression of severe coronary artery disease in patients with variation in the MBL gene[44]; and as homozygosity for *MBL2* variant alleles is associated with greater impairment in flow mediated dilatation during infection in childhood[24], it could be hypothesized that deficiency of MBL in SLE patients results in defective clearance of infectious material that aggravates ongoing inflammatory processes leading to development of atherosclerosis.

Endothelial apoptosis contributes to the loss of endothelial integrity and thereby to the initiation of atherosclerosis[45]. In patients with SLE the number of apoptotic circulating endothelial cells is increased, which correlates with the endothelial dysfunction observed in these patients[46]. Moreover, SLE patients produce a heterogeneous array of antibodies that bind to the endothelium[47,48] and *in vitro* studies have shown that some of these antibodies induce endothelial apoptosis[49]. Lack of functional MBL may lead to decreased removal of apoptotic endothelial cells in patients with SLE. As high levels of circulating apoptotic endothelial cells may induce production of autoantibodies directed against endothelial cells, this could lead to dysfunction of the endothelium and enhance development of atherosclerosis in patients with SLE.

In contrast to our observations in SLE patients, the normal *MBL2* genotype and high serum levels of MBL have also been shown to increase the risk of CVD in apparently healthy men[50], in patients with rheumatoid arthritis (RA)[51], and in patients with diabetes[52]; and high serum levels of MBL and the lectin pathway of complement initiates the inflammatory reaction seen in relation to ischemia reperfusion[53,54]. A differentiated role of MBL in atherosclerosis development was recently observed in patients with RA[55]. This paradox is probably a key issue in understanding the role of MBL in human disease. Thus, it is likely that a fine balance in the vessel wall during all stages of development of atherosclerosis determines whether MBL may be advantageous or disadvantageous. Under circumstances allowing the physiological opsonic activity of MBL, which is advantageous, lack of functional MBL may increase inflammation due to dysfunctional sequestration of harmful material from the vessel wall. On the other hand, if high levels of MBL mediate excessive complement activation the process may become detrimental. However, based on our results we are not able to determine causal relations. Experimental studies should be performed to examine the mechanisms by which MBL may influence the various stages of atherosclerosis in SLE patients and in other study populations.

Our results support the notion that genetically determined low levels of MBL are associated with the development of atherosclerotic disease in SLE. As our study population was relatively small, new studies based on larger study populations should be performed to confirm our data and to examine if our findings may be applicable in other disease settings and in healthy individuals.

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