

# Levels in Plasma of the Serine Proteases and Associated Proteins of the Lectin Pathway Are Altered in Patients with Systemic Lupus Erythematosus

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**ABSTRACT. Objective.** To examine whether proteins of the lectin pathway of the complement system are involved in systemic lupus erythematosus (SLE) pathogenesis.

**Methods.** Using time-resolved immunofluorometric assays, plasma levels of mannan-binding lectin (MBL)-associated serine proteases 1 (MASP-1), MASP-2, MASP-3, MBL-associated protein of 19 kDa (MAp19), and MAp44 were determined in 58 patients with SLE and 65 healthy controls (HC).

**Results.** Plasma concentrations in patients with SLE were higher than HC regarding MASP-1, MASP-3, and MAp44 ( $p < 0.0001$ ,  $0.0003$ , and  $0.0013$ ). Complement factor 3 correlated negatively and anti-dsDNA positively with levels of MAp19 ( $p = 0.0035$ ,  $p = 0.0133$ ).

**Conclusion.** In SLE, plasma levels of MASP and MAp are altered and associated with SLE characteristics, supporting a role in SLE pathogenesis. (First Release April 15 2015; J Rheumatol 2015; 42:948–51; doi:10.3899/jrheum.141163)

## Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS  
LECTIN PATHWAY

COMPLEMENT SYSTEM  
INNATE IMMUNITY

Systemic lupus erythematosus (SLE) is a complex autoimmune disease involving both the innate and the adaptive immune systems, but where the pathogenesis is still not fully understood<sup>1</sup>. However, the innate immune system and in particular the complement system is believed to play a key role. Measurements of the level of complement proteins (e.g., C3 and C4) are used in monitoring SLE disease activity<sup>2</sup>. The strongest known monogenic association to SLE is defects in the *C1q* gene, confirming the importance of the complement system in SLE<sup>3</sup>. The complexity of the pathogenic mechanisms regarding complement is underlined when viewing the classical pathway of the complement system. The immune complexes that deposit in the kidney

effectively activate the complement system through the classical pathway<sup>4</sup>. Yet deficiency of complement C1q, which is necessary for classical pathway activation, leads to SLE in more than 90% of cases<sup>3</sup>. This seems like a paradox, because complement is thought to contribute to the damage seen in SLE<sup>5</sup>. However, it is possible that complement activation acts as a double-edged sword, being highly important in preventing SLE and exacerbating it once the disease has been established<sup>6</sup>.

The lectin pathway is one of 3 ways to activate the complement system<sup>7</sup>. Activation takes place when one of the 6 pattern recognition molecules [PRM; mannan-binding lectin (MBL), M-ficolin, L-ficolin, H-ficolin, Collectin-L1, or Collectin-K1] binds to a surface recognized by the PRM. The PRM form complexes with MBL-associated serine proteases (MASP) that then become active enzymes, leading to the initiation of the common pathway<sup>8</sup>. The gene *MASP1* encodes MASP-1, MASP-3, and MBL-associated protein of 44 kDa (MAp44), whereas the gene *MASP2* encodes MASP-2 and MAp19. MASP-1 and MASP-2 are directly involved in the complement activation while MAp44 and MAp19 are thought to have a regulatory function<sup>9,10</sup>. MASP-3's enzymatic function and role in the activation of the complement system is not clear<sup>11</sup>.

Proteins of the lectin pathway have been implicated to play a role in the pathogenesis of SLE. MBL has been studied most elaborately with somewhat conflicting results<sup>12</sup>. However, the functional *MBL2* codon 54 polymorphism was associated with SLE susceptibility<sup>13</sup>. This is supported by the

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association of MBL deficiency and SLE becoming stronger with accompanying complement deficiency<sup>14</sup>.

Regarding studies of H-, L-, and M-ficolin, they all have associations with signs of disease activity in SLE, but also with conflicting results<sup>15,16</sup>. No studies have focused on MAP or MASP in relation to SLE.

The objective of this pilot study was to measure the plasma concentrations of the MASP and MAP of the lectin pathway in SLE, and to compare the concentrations to a group of age- and sex-matched healthy controls (HC). A further objective was to analyze for associations between the plasma concentrations and SLE disease activity score and characteristic SLE manifestations.

## MATERIALS AND METHODS

**Patients.** Plasma was obtained from a cross-sectional cohort of 58 female patients with SLE. All patients were recruited at the outpatient clinic at the Department of Rheumatology, Aarhus University Hospital. We prospectively collected demographic and clinical data using the SLE Disease Activity Index score<sup>2</sup> and organ damage data using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (ACR) Damage Index<sup>17</sup>. Inclusion criteria were age above 18, female, and the fulfillment of the 1997 revised ACR classification criteria for SLE<sup>18</sup>. No incapacitated patients were included (Table 1). For comparison, blood samples were collected from 65 age- and sex-matched HC.

**Methods.** Peripheral venous blood samples were collected in cell preparation tubes (BD Vacutainer, BD Diagnostics) and processed within 1 h. Blood samples were centrifuged at room temperature for 30 min at 1800 g, and plasma was divided and stored at  $-80^{\circ}\text{C}$ .

Plasma levels of MASP-1<sup>19</sup>, MASP-2<sup>20</sup>, MASP-3<sup>9</sup>, MAP19<sup>10</sup>, and MAP44<sup>9</sup> were measured using time-resolved immunofluorometric in duplicates. All assays and specific antibodies for the assays were produced in-house. Detailed description for each assay can be found in the references for each protein<sup>9,10,19,20</sup>.

**Statistics.** The Mann-Whitney U test was used for comparison of plasma levels of the proteins in patients and controls, and correlation analysis was performed calculating Spearman rank correlation coefficient and logistic regression analysis. P values  $< 0.05$  were considered statistically significant.

**Ethics statement.** The Danish Data Protection Agency and The Regional Committee on Health Research Ethics approved the study (case no. 1-10-72-214-13). Clinical investigations were conducted according to the Declaration of Helsinki.

## RESULTS

The cohort in our study was compatible with usual European SLE cohorts with the exception of a slightly smaller number of patients with kidney affection (Table 1).

Plasma levels of the proteins encoded by the *MASP1* gene (MASP-1, MASP-3, and MAP44) were all higher in the SLE group than in HC ( $p < 0.0001$ ,  $0.0003$ , and  $0.0013$ ). For the proteins encoded by the *MASP2* gene (MASP-2 and MAP19), patients with SLE had a lower plasma level of MAP19 than HC ( $p < 0.0001$ ; Table 2, and Supplementary Figure 1, available online at jrheum.org).

For patients who previously had nephritis, MASP-3 plasma levels were lower than patients who never had a history of nephritis ( $p = 0.0409$ ; Table 3). Patients with current anemia had lower plasma levels of MASP-2 than

Table 1. Demographics and clinical characteristics for patients with SLE and HC. Values are n (%) or mean  $\pm$  SD unless otherwise specified.

Characteristics	Patients, n = 58
M/F	0/58
Age at diagnosis, yrs, mean $\pm$ SD (range)	32 $\pm$ 12.3 (14–64)
Age at inclusion, yrs, mean $\pm$ SD (range)	46.1 $\pm$ 13.1 (24–69)
Danish ethnicity	58 (100)
Hydroxychloroquine treatment	45 (78)
Prednisolone treatment	22 (38)
Mycophenolate mofetil	6 (10)
Azathioprine	10 (17)
No treatment	6 (10)
ACR criteria	
Malar rash, ACR-1	27 (46.6)
Discoid rash, ACR-2	11 (19.0)
Photosensitivity, ACR-3	28 (48.3)
Oronasal ulcers, ACR-4	13 (22.4)
Arthritis, ACR-5	46 (79.3)
Serositis, ACR-6	14 (24.1)
Nephritis, ACR-7	7 (12.1)
CNS, ACR-8	4 (6.89)
Hematological, ACR-9	33 (57.0)
Immunological, ACR-10	46 (79.3)
ANA, ACR-11	57 (98.3)
Present anti-dsDNA level, $\times 10^3$ IU/l	33.3 $\pm$ 15.7
Disease activity/organ damage	
SLEDAI	6.48 $\pm$ 4.03
SDI	1.72 $\pm$ 1.56
HC characteristics	
M/F	0/65
Age, yrs, mean $\pm$ SD (range)	44.03 $\pm$ 12.2 (21–64)

SLE: systemic lupus erythematosus; HC: healthy controls; ACR: American College of Rheumatology; CNS: central nervous system; ANA: antinuclear antibody; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/ACR Damage Index.

patients without anemia ( $p = 0.0194$ ), but when analyzing for correlation between hemoglobin level and MASP-2 level in plasma, no correlation was observed. For MAP19, patients with low C3 levels and patients with a positive anti-dsDNA titer had higher plasma levels of MAP19 than patients with normal C3 levels and patients with negative anti-dsDNA titer ( $p = 0.0015$  and  $p = 0.026$ , respectively). Interestingly, there was a negative correlation between plasma levels of C3 and MAP19 ( $r = -0.3907$ ,  $p = 0.0035$ ) and a positive correlation between levels of anti-dsDNA and MAP19 ( $r = 0.3289$ ,  $p = 0.0133$ ; Supplementary Figure 2 available online at jrheum.org). Complete results for each protein are shown in Supplementary Tables available at jrheum.org.

No statistically significant differences were seen between protein concentrations in patients receiving immunosuppressing treatment ( $n = 52$ ) compared with those who did not ( $n = 6$ ).

## DISCUSSION

The plasma concentrations of several of the MASP and MAP were significantly altered in a cross-sectional cohort of

Table 2. Plasma concentrations of MASP and MAp in patients with SLE and HC.

Results	Patients with SLE, n = 58, ng/ml, Median (range)	HC, n = 65, ng/ml, Median (range)	Difference Between Medians, Hodge-Lehman, ng/ml	Mann-Whitney p
MASP-1	10,341 (5554–29,683)	6929 (1771–19,716)	3557	< 0.0001
MASP-3	4902 (2856–9910)	4041 (1624–7769)	908	0.0003
MAp44	4412 (1228–5527)	1938 (1250–3836)	380	0.0013
MASP-2	260 (55–772)	242 (95–524)	19	0.2710
MAp19	163 (22–442)	249 (64–583)	74	< 0.0001

MASP: mannan-binding lectin-associated serine proteases; MAp: mannan-binding lectin-associated protein; SLE: systemic lupus erythematosus; HC: healthy controls; MAp44: mannan-binding lectin-associated protein of 44 kDa.

Table 3. Differences in plasma levels of MASP and MAp in patients with and without characteristic manifestations of SLE. Values are median (range) unless otherwise specified.

Manifestations	Patients with Manifestations		Patients without Manifestations		p
	No. Patients	MASP-1	No. Patients	MASP-1	
SDI-positive vs -negative	43	10,512 (5554–29,684)	15	8060 (6225–16,590)	0.0195
Nephritis	7	MASP-3	51	MASP-3	0.0409
		MASP-2		MASP-2	
Anemia	9	MASP-2	49	MASP-2	0.0194
		MAp19		MAp19	
Low C3 count	25	182 (74–442)	33	129 (22–290)	0.0015
Anti-dsDNA-positive vs -negative	34	171.5 (22–442)	24	122 (30–295)	0.026

Positive SDI score is a score > 0. Anemia = plasma hemoglobin < 7.3 mmol/l. Measurement of anti-dsDNA was considered positive > 10 × 10<sup>3</sup> IU/l. MASP: mannan-binding lectin-associated serine proteases; MAp: mannan-binding lectin-associated protein; SLE: systemic lupus erythematosus; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; C3: complement factor 3; MAp19: MBL-associated protein of 19 kDa.

patients with SLE showing high levels of MASP-1, MASP-3, and MAp44, and low levels of MAp19. Further, we observed a clear difference in plasma levels of MASP-3 in patients with SLE who had nephritis compared to the patients without, and that MAp19 displays a negative correlation with the levels of C3 and a positive correlation with the levels of anti-dsDNA.

Within the innate immune system, deficiencies in the complement system are already described to have an association with SLE. This makes pattern recognition within the lectin pathway a major interest with regard to SLE.

The difference in plasma levels of MASP-3 in patients with SLE who had nephritis compared with the patients without is notable because glomerulonephritis is a major cause of organ damage in patients with SLE. It is possible that MASP-3 plays a specific pathogenic role in patients with SLE with nephritis. This influence is maybe also reflected in the difference between MASP-3 concentrations in the SLE cohort and healthy individuals, where higher levels of MASP-3 are seen in the patients.

An intriguing result is that MAp19 displays a negative correlation with the levels of C3 and a positive correlation with the levels of anti-dsDNA.

Low plasma levels of C3 in combination with high plasma

levels of anti-dsDNA are core elements in the immunological diagnosis of SLE, and changes in these variables are useful laboratory values to monitor disease activity.

For other SLE manifestations, there were no significant difference in plasma concentrations in MAp19 between patients with and without the manifestations. This is not surprising, because the clinical manifestations could have been present at any time, whereas C3 and anti-dsDNA were measured in the same blood that we used to measure the lectin pathway proteins, and thus represents the patient's current immunological status best.

The biological functions of both MASP-3 and MAp19 are far from completely clarified<sup>8,11</sup>, making interpretations of the changes seen in patients with SLE complicated. The low levels we observed could result from the antibodies against the proteins, consumption, or they could be genetically determined. It is proposed that MASP-3 attenuates the lectin pathway activity because of competition for MASP-binding sites<sup>9</sup>. In this case, higher levels seen in SLE make sense, to inhibit activation of complement. Hein, *et al's* results support such an interpretation<sup>16</sup> because elevated levels of H-ficolin in patients with SLE were without difference in the H-ficolin-mediated lectin pathway activity in patients with SLE.

Seven patients (12%) had kidney involvement. In our rheumatologic outpatient clinic, Aarhus University Hospital, about 18% of patients with SLE have been or are undergoing treatment for nephritis. The smaller number of patients with nephritis in our study could be a matter of chance, or it could represent a possible selection bias.

Only female patients were included in this project, because the project is part of a genetic study; The Regional Committee on Health Research Ethics argued that anonymity for the male patients could not be ensured if male patients were included in a group of 60 patients with SLE.

The lectin pathway proteins in the pathological mechanisms of SLE in our study call for additional investigation. It is, however, clear that the lectin pathway in patients with SLE is associated with key manifestations of the disease, supporting a central role in SLE.

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#### ONLINE SUPPLEMENT

Supplementary data for this article are available online at [jrheum.org](http://jrheum.org).

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