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Collectin liver 1 and collectin kidney 1 and other complement-associated pattern recognition molecules in systemic lupus erythematosus

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Summary

The objective of this study was to explore the involvement of collectin liver 1 (CL-L1) and collectin kidney 1 (CL-K1) and other pattern recognition molecules (PRMs) of the lectin pathway of the complement system in a cross-sectional cohort of systemic lupus erythematosus (SLE) patients. Concentrations in plasma of CL-L1, CL-K1, mannan-binding lectin (MBL), M-ficolin, H-ficolin and L-ficolin were determined in 58 patients with SLE and 65 healthy controls using time-resolved immunoflourometric assays. The SLE patients' demographic, diagnostic, clinical and biochemical data and collection of plasma samples were performed prospectively during 4 months. CL-L1, CL-K1 and M-ficolin plasma concentrations were lower in SLE patients than healthy controls (P-values $< 0{\cdot}001$, $0{\cdot}033$ and $< 0{\cdot}001$, respectively). H-ficolin concentration was higher in SLE patients ($P < 0{\cdot}0001$). CL-L1 and CL-K1 plasma concentrations in the individuals correlated in both patients and controls. Patients with low complement component 3 (C3) demonstrated a negative correlation between C3 and CL-L1 and CL-K1 ($P = 0{\cdot}022$ and 0.031, respectively). Patients positive for anti-dsDNA antibodies had lower levels of MBL in plasma than patients negative for anti-dsDNA antibodies ($P = 0{\cdot}02$). In a cross-sectional cohort of SLE patients, we found differences in the plasma concentrations of CL-L1, CL-K1, M-ficolin and H-ficolin compared to a group of healthy controls. Alterations in plasma concentrations of the PRMs of the lectin pathway in SLE patients and associations to key elements of the disease support the hypothesis that the lectin pathway plays a role in the pathogenesis of SLE.

Keywords: complement system, lectin pathway, pattern recognition molecules, systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, which affects primarily women of childbearing age, and disease progression involves both the innate and the adaptive immune system. It is a severe systemic disease potentially affecting multiple organ systems, but the pathogenesis remains unresolved [1]. However, several studies indicate that the innate immune system, and complement in particular, plays a key role. In the daily clinic, measurements of complement proteins (e.g. C3 and C4) are used in monitoring SLE disease activity [2] and complement component 1q (C1q) plays a role in the pathogenesis, as deficiency is associated strongly with SLE [3–5].

The complement system is part of the innate immune defence. It comprises three initiating pathways: the classical, alternative and lectin pathways [6,7]. The latter pathway activates the complement system when one of six pattern recognition molecules (PRMs) – mannan-binding lectin (MBL), M-ficolin, L-ficolin, H-ficolin, collectin liver 1 (CL-L1) or collectin kidney 1 (CL-K1), in complex with the MBL-associated serine proteases (MASPs) – bind to a surface recognized by the PRMs. The MASPs then become enzymatically active and initiate the common pathway of the complement system [8]. This activation results in opsonization of the target, chemotaxis of leucocytes and lysis of the recognized cell [6].

CL-L1 and CL-K1 are the last members of the PRMs of the lectin pathway to be discovered [9,10]. Both PRMs are found in circulation with MASPs [11,12], and it was revealed recently that most of the CL-K1 exists in serum in the form of heteromeric complexes with CL-L1, making up a CL–LK heterocomplex. The heteromeric complex, CL–LK, interacts avidly with MASPs and mediates complement activation potently in comparison with, e.g. CL-K1 alone [11,12]. In the present work we have chosen to detect both of the two different polypeptide chains (CL-L1 and CL-K1 polypeptide chains) of the complex and make statistical analyses separately for the concentrations of each of them. The preceding literature describes examinations of the protein as if there are two different molecules, and CL-K1 was shown to bind carbohydrate moieties such as fucose and mannose and CL-L1 to bind mannose, *N*-acetylglucosamine, galactose and fucose [9,10,12,13]; more recently, the recognition domain of the CL-K1 polypeptide chain was described in complex with a di-mannose structure [14]. As is also the case for the other soluble pattern recognition molecules mentioned above, a very low affinity between the single recognition domain and, for example, monosaccharides is seen. Only when clustering of the domains through oligomerization happens will there be a high avidity binding towards patterns of carbohydrate structures. Using the CL-K1 polypeptide chain alone, it was also found that an activation of complement could be observed [15].

CL-K1 binds DNA-coated surfaces, which leads to C4b-deposition via MASPs, and could thus play a role in response to particles and surfaces presenting extracellular DNA, such as apopototic cells, neutrophil extracellular traps and biofilms [16]. The concentration of the two polypeptide chains, CL-L1 and CL-K1, is found in closely associated concentrations, again indicating that they exist as one complex, CL–LK [17].

Five different mutations have been identified in the gene encoding CL-K1 (*COLEC11*) associated with the severe developmental 3MC syndrome [18]. A recent clinical study observed higher plasma concentrations of CL-K1 in patients with disseminated intravascular coagulation [19].

There is considerable evidence that defects in the removal of apoptotic cells is associated with autoimmune disease [20,21]. PRMs of the lectin pathway appear to assist in disposing of dying host cells by binding apoptotic cells, promoting macropinocytosis of the debris [22–24]. Accordingly, MBL-deficient mice also clear apoptotic cells poorly [25].

MBL have been implicated in the pathogenesis of SLE with conflicting results [21,26]. However, the polymorphism in the *MBL2* gene at codon 54, which gives rise to an amino acid change resulting in low MBL concentrations in plasma, has been associated with SLE susceptibility [27]. A more pronounced association of MBL deficiency and SLE has also been seen in patients with accompanying complement deficiency [28]. Further, anti-MBL antibodies are present in sera from SLE patients and influence the functional activity of MBL [29]. There have been only few reports addressing the ficolins in relation to SLE. High serum concentrations of H-ficolin and low L-ficolin concentrations have been measured in serum from SLE patients [30,31], but only the high H-ficolin levels were confirmed in a recent report [32]. To our knowledge, there are no publications on CL-K1 or CL-L1 in SLE.

The aim of this pilot study was to assess the plasma concentrations of CL-K1, CL-L1 and the remaining PRMs of the lectin pathway in patients diagnosed with SLE and in age- and gender-matched healthy controls. Further, we analysed for associations between the PRMs of the lectin pathway and disease manifestations in SLE.

Materials and methods

Patients

From a cross-sectional cohort of 58 SLE patients, we collected prospectively plasma samples, demographic, biochemical and clinical data including American College of Rheumatology (ACR) classification criteria [33], SLE disease activity index score (SLEDAI) [2] and organ damage using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) [34]. The inclusion criteria were: fulfilment of the 1997 revised ACR classification criteria for SLE [33], female, aged more than 18 years and exclusion of incapacitated patients. For comparison, plasma samples were collected from 65 age- and gender-matched healthy blood donors (Table 1). Only females were included, as it would not be possible to ensure anonymity for males in a group of 58 SLE patients.

Methods

CPT™ tubes (BD Vacutainers®; BD Diagnostics, Franklin Lakes, NJ, USA) were used to collect peripheral venous blood samples. The time from collection to processing the blood did not exceed 1 h. Blood samples were centrifuged at 1800 ***g*** for a minimum of 30 min at room temperature in a horizontal rotor, aliquoted and then stored at −80°C.

Plasma concentrations of CL-L1 [35], CL-K1 [36], MBL [37], M-ficolin [38] and H-ficolin [39] were determined using time-resolved immunofluorometric assays (TRIFMA). The assays and specific antibodies for CL-K1, CL-L1, MBL, M-ficolin and H-ficolin were produced in-house, and for each protein a detailed description for the assays can be found within the references given above [35–39]. We estimated L-ficolin plasma concentrations using an enzyme-linked immunosorbent assay (ELISA) (Hycult Biotech®, Uden, the Netherlands), according to the manufacturer's instructions.

Table 1. Demographics and clinical characteristics of systemic lupus erythematosus (SLE) patients and demographics of healthy controls

Characteristics	Patients ($n = 58$)
M/F	0/58
Mean age at diagnosis ± s.d. (range)	32 ± 2·3 (14–64)
Mean age at inclusion ± s.d. (range)	46·1 ± 13·1 (24–69)
Ethnic Danes (%)	58 (100)
Hydroxychloroquine treatment *n* (%)	45 (78)
Prednisolone treatment *n* (%)	22 (38)
Mycophenolate mofetil *n* (%)	6 (10)
Azathioprine *n* (%)	10 (17)
No treatment *n* (%)	6 (10)
ACR criteria	
Malar rash (ACR-1) *n* (%)	27 (46·6)
Discoid rash (ACR-2) *n* (%)	11(19·0)
Photosensitivity (ACR-3) *n* (%)	28 (48·3)
Oronasal ulcers (ACR-4) *n* (%)	13 (22·4)
Arthritis (ACR-5) *n* (%)	46 (79·3)
Serositis (ACR-6) *n* (%)	14 (24·1)
Nephritis (ACR-7) *n* (%)	7 (12·1)
CNS (ACR-8) *n* (%)	4 (6·89)
Haematological (ACR-9) *n* (%)	33 (57·0)
Immunological (ACR-10) *n* (%)	46 (79·3)
ANA (ACR-11) *n* (%)	57 (98·3)
Present anti-ds-DNA level ($*10^3$ IU/l) ± s.d.	33·3 ± 15·7
Disease activity/organ damage	
SLEDAI mean ± s.d.	6·48 ± 4·03
SDI mean ± s.d.	1·72 ± 1·56
Characteristics healthy controls	
M/F	0/65
Mean age ± s.d. (range)	44·03 ± 12·2 (21–64)

ANA = anti-nuclear antibody; CNS = central nervous system; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLEDAI = SLE disease activity index; ACR = American College of Rheumatology; M/F = male/female; s.d. = standard deviation.

In brief, samples were thawed at 4°C overnight and prediluted fourfold in Tris-buffered saline (10 mM Tris, 145 mM NaCl, 15 nM NaN_2 pH 7·4) and thereafter freeze–thaw cycles were kept at a minimum. The TRIFMA assays are traditional sandwich immunoassays using a combination of two monoclonal antibodies; one is used to coat the microtitre plate, the other antibody is biotinylated and used to detect the antigen bound to the coating antibody. Europium-labelled streptavidin, which binds biotin, is added, and the europium is detected after the addition of enhancement solution on a fluorometer (Victor X5®; PerkinElmer, Waltham, MA, USA). All samples were added in duplicate and the analysis was repeated if the coefficient of variation (%CV) was greater than 20% between the two wells. For all assays, internal controls were used to ensure reproducibility and plates were repeated if the quality controls varied with %CV > 15 compared with our laboratory standard values. Regarding the concentrations given for CL-L1, for the present report we determined the concentration of CL-L1 in standard serum using a new and better-characterized preparation of recombinant CL-L1, resulting in levels approximately three times lower than given in our previous report [35].

Statistics

The data were checked for normality by Q–Q plots and histograms, and Gaussian distribution could not be assumed. Therefore, non-parametric tests were used for the statistical analysis. 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's rank correlation coefficient. *P*-values < 0·05 were considered statistically significant. Stata® version 12 and GraphPad Prism® software package (version 6·0) were used for data management and statistical calculations.

Ethics statement

The Regional Committee on Health Research Ethics (case no. 1-10-72-214-13) and the Danish data protection agency approved the study. The project was performed in pursuance of the Helsinki Declaration.

Results

The study cohort was comparable with other European SLE cohorts, except for a lower number of patients with kidney infection (Table 1).

The concentrations of CL-L1, CL-K1 and M-ficolin in plasma were found to be lower in patients than in healthy controls (*P*-values < 0·0001, < 0·0334 and < 0·0001, respectively). The median concentration of H-ficolin in patients was significantly higher than in healthy controls. We observed no statistically significant difference between median concentration of MBL and L-ficolin in SLE patients and healthy controls (Table 2 and Supporting information, Fig. S1).

A positive correlation ($r = 0·576$, $P < 0·0001$) was found between the concentrations of CL-K1 and CL-L1 in plasma in both patients and in healthy controls (Fig. 1).

For patients with discoid rash we observed higher plasma concentrations of CL-L1, M-ficolin and H-ficolin compared to patients without this manifestation (Table 3). In patients with mucosal ulcers or lymphopenia, we also observed higher H-ficolin concentrations in plasma compared to patients who did not display these symptoms. In the patients with lymphopenia, there was a positive correlation between lymphocyte count and H-ficolin concentration, although it did not reach statistical significance (Table 4).

Patients who were positive for anti-dsDNA antibodies showed lower levels of MBL than anti-dsDNA antibody-negative patients ($P = 0·020$). A negative correlation was found between concentrations of both CL-L1 and CL-K1 and C3 concentration in patients with low C3 concentrations (Table 4 and Fig. 2).

Table 2. Plasma concentrations of the pattern recognition molecules of the lectin complement pathway in systemic lupus erythematosus (SLE) patients and healthy controls

Results	SLE patients (n = 58) Median plasma conc. μg/ml (range)	Healthy controls (n = 65) Median plasma conc. μg/ml (range)	Mann–Whitney P-value
CL-L1	0·25 (0·16–0·37)	0·31 (0·20–0·51)	<0·001
CL-K1	0·33 (0·22–0·45)	0·34 (0·25–0·50)	0·033
MBL	0·75 (0·00–3,25)	0·70 (4–4,203)	0·520
M-ficolin	0·30 (0·01–1·01)	0·49 (0·16–1·63)	<0·001
L-ficolin	2·50 (1·09–6·11)	2·40 (0·32–6·44)	0·612
H-ficolin	21·5 (6·64–53·9)	16·0 (5·17–25·3)	<0·001

CL-L1 = collectin liver 1; CL-K1 = collectin kidney 1; MBL = mannan-binding lectin.

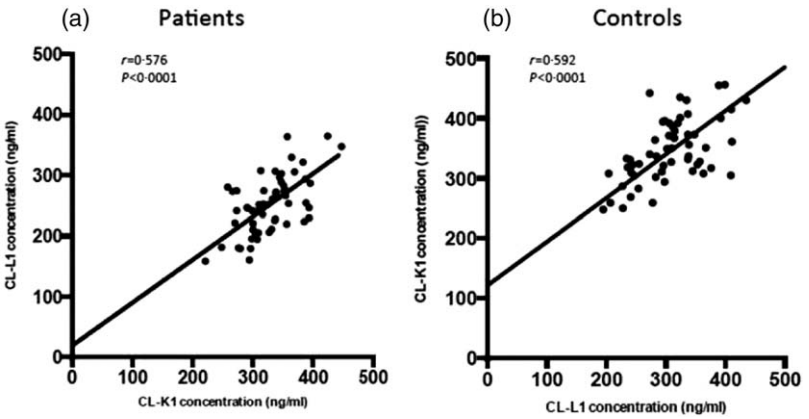

Fig. 1. Correlation of collectin liver 1 (CL-L1) and collectin kidney 1 (CL-K1) plasma concentration in systemic lupus erythematosus (SLE) patients (a) and healthy controls (b).

Table 3. Differences in plasma concentrations of the pattern recognition molecules in patients with and without characteristic manifestations of systemic lupus erythematosus (SLE)

Patients with manifestations			Patients without manifestations		
Manifestation	n		n		P
		CL-L1 median (range)		CL-L1 median (range)	
Discoid rash	11	0·281 (0·161–0·330)	47	0·247 (0·159–0·365)	0·036
		M-ficolin median (range)		M-ficolin median (range)	
Discoid rash	11	0·444 (0·098–0·878)	47	0·228 (0·010–1·013)	0·028
		H-ficolin median (range)		H-ficolin median (range)	
Discoid rash	11	31·3 (15·8–42·8)	47	20·5 (6·64–54·0)	0·012
Lymphopenia	30	24·7 (12·8–54·0)	28	20·3 (6·64–40·7)	0·022
Mucosal ulcers	13	31·3 (13·3–54·0)	45	20·5 (6·64–51·1)	0·037
		MBL median (range)		MBL median (range)	
Anti-dsDNA-positive	34	0·311 (0·003–2·265)	24	0·970 (0·010–3·249)	0·020

Lymphopenia was determined as blood counts $< 1{\cdot}30 \times 10^9$/l. Measurement of anti-ds-DNA were considered positive $>10 \times 10^3$ IU/l. CL-L1 = collectin liver 1.

No associations were found between disease activity (SLEDAI) or accumulated organ damage (SDI score) with concentrations in plasma of the PRM (Supporting information,Table S1).

Discussion

Adaptive immunity has been the main focus of research in SLE, particularly in the attempts to develop new therapies for the disease. Intuitively, this makes sense, as autoantibodies and immune complexes, which attack the patient's own tissue, characterize the disease [40]. However, B cell-depleting therapy, targeting the antibody-producing cells, have had disappointing clinical efficacy in active SLE [41–43]. These observations have led to a renewed interest in the potential role of the innate immune system in the pathogenesis of SLE.

In the innate immune system, deficiencies in the complement system have already been associated with SLE [1], and this makes pattern recognition within the lectin pathway interesting.

Table 4. Spearman's rank correlation between key laboratory findings in the systemic lupus erythematosus (SLE) patients and plasma concentrations of the pattern recognition molecules

	Concentration											
	CL-L1		CL-K1		MBL		M-ficolin		L-ficolin		H-ficolin	
	rho	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*
Lymphopenia ($n = 30$)	−0·128	0·500	−0·203	0·281	−0·298	0·109	0·116	0·542	0·406	0·055	0·353	0·056
Anti-ds-DNA-positive ($n = 34$)	0·281	0·120	0·303	0·091	0·125	0·496	−0·275	0·127	−0·097	0·624	0·018	0·923
Low C3 ($n = 25$)	−0·464	0·022	−0·442	0·031	−0·014	0·941	0·002	0·992	0·199	0·386	0·067	0·750

Lymphopenia was determined as blood counts $< 1{\cdot}30 \times 10^9$/l. Measurement of anti-ds-DNA was considered positive $> 10 \times 10^3$ IU/l. Low C3 was determined as plasma concentrations <0·90 g/l. CL-L1 = collectin liver 1; CL-K1 = collectin kidney 1; MBL = mannan-binding lectin.

In this cross-sectional cohort of SLE-patients, we found plasma concentrations of several PRMs of the lectin pathway to be altered significantly, compared to healthy controls with low levels in plasma of CL-L1, CL-K1 and M-ficolin and high levels of H-ficolin in SLE patients.

The clear correlation between CL-L1 and CL-K1 substantiates the findings by Henriksen *et al.* [16] that the two proteins form a heteromeric complex (CL–LK) and thereby exist in more or less equal amounts in the circulation. It is noteworthy that, in the present report, the levels of CL-L1 for the healthy individuals are lower than we reported originally for blood donors [35], as we have now managed to develop a better quantification of the CL-L1 content in our standard serum. Further, both proteins are lower in SLE patients than healthy controls, and there is a negative correlation between the plasma concentrations of both CL-L1 and CL-K1 and C3 in patients with low C3. Whether the low levels of CL-L1 and CL-K1 in SLE patients in our study are caused by polymorphisms in the genes encoding the proteins [17], due to consumption because of increased activation of the lectin pathway, antibodies against the proteins or faster clearance of the proteins due to binding to apoptotic cells, remains to be investigated.

It would seem paradoxical that we observe lower concentrations of CL-K1 and CL-L1 in SLE patients compared to healthy controls. However, when the analysis is confined to the subgroup of patients with low C3, in general the patients with the highest disease activity, they have higher levels of CL-L1 and CL-K1. The same complexity is seen when viewing the classical pathway of the complement system. The immune complexes that deposit in the kidney effectively activate the complement system via the classical pathway [44]. However, deficiency of complement C1q, which is necessary for classical pathway activation, leads to SLE in more than 90% of cases [3]. This seems to be a paradox, as complement is thought to contribute to the damage seen in SLE [45]. 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 [44].

For MBL, we did not see a difference in plasma concentrations between patients and healthy controls. We did, however, observe a very clear difference in MBL concentrations between SLE patients who were positive for anti-dsDNA antibodies and those who were not, indicating a potential pathogenic role of MBL in the subset of SLE patients who are positive for anti-dsDNA antibodies. The genetic association of low MBL with SLE [27] would probably have been stronger if only patients positive for anti-dsDNA antibodies were examined.

High plasma concentrations of H-ficolin in SLE patients and an association to lymphopenia have been described previously [30,32], and Ucieklak *et al.* described an association between high levels of H-ficolin and SLE glomerulonephritis. We could not confirm the association between glomerulonephritis and H-ficolin, but this could be

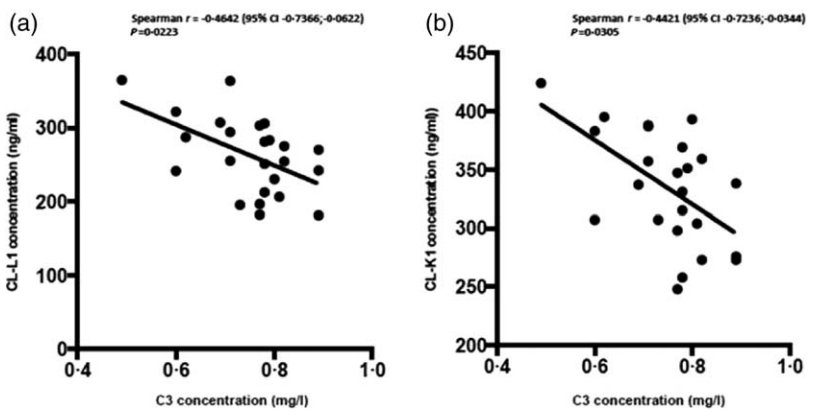

Fig. 2. Correlation between C3-plasma concentrations and collectin liver 1 (CL-L1) (a) and collectin kidney 1 (CL-K1) (b) in systemic lupus erythematosus (SLE) patients with low C3-count (low = C3 < 0·90 mg/l).

explained by the relatively small number of patients in the subgroups. In our SLE cohort, 12% of patients had kidney involvement. Approximately 18% of approximately 200 SLE patients in our out-patient clinic are treated for nephritis. This could possibly represent a selection bias, or could be due to chance.

We found that SLE patients with discoid skin manifestations had higher plasma concentrations of CL-L1, M-ficolin and H-ficolin than patients without discoid lesions. Recently, Wallim *et al.* [46] described MBL depositions in lesional skin of SLE patients, but not in non-lesional skin or in discoid skin lesions. Our findings support a different pathogenic mechanism in the discoid skin manifestation of SLE and indicate an association with the lectin pathway proteins.

Deciphering the complex interaction of the lectin pathway proteins in the pathological mechanisms of SLE is a challenging task; the limitations posed by the small patient subgroups in the present study are obvious, and call for additional investigations in large SLE cohorts. However, it appears that there are significant changes in the PRMs of the lectin pathway in SLE patients and that the associations with key manifestations of the disease found in our study indicate a pathogenic role in SLE.

In conclusion, we observed significant concentration changes in plasma of the PRMs of the lectin pathway in SLE patients compared to healthy controls and found significant associations with central manifestations of SLE. Our findings support a pathogenic role of the lectin pathway in SLE.

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Author contributions

A. T. and L. J. performed the laboratory experiments; A. T., B. D. and K. S. was in charge of collecting clinical data and blood samples; S. H., S. T. and J. C. J. developed the assays used in the project and supervised laboratory procedures; M. J. L. handled the blood samples after they were drawn and collected and handled the control material. A. T., S. T. and K. S. wrote the manuscript and all authors participated in the editing of the article.

Disclosure

The authors declare no conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Scatterplots of the plasma concentration of all the pattern recognition molecules of the lectin pathway in patients and healthy controls.

Table S1. Spearman's rank correlation between disease activity score, organ damage score and plasma concentrations of the pattern recognition molecules