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Ficolins and the lectin pathway of complement in patients with systemic lupus erythematosus☆

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ABSTRACT

The complement system plays a pathophysiological role in systemic lupus erythematosus (SLE). This study aims to investigate whether an association exists between the ficolins that are part of the lectin complement pathway and SLE.

EDTA plasma samples from 68 Danish SLE patients and 29 healthy donors were included in the study. Plasma concentrations of Ficolin-1, -2, and -3 were determined in specific sandwich ELISAs. Lectin pathway activity via Ficolin-3 was measured in ELISA on acetylated bovine serum albumin (acBSA) and measured as Ficolin-3 binding and deposition of C4, C3 and the terminal complement complex (TCC).

SLE patients had increased levels of Ficolin-3, 21.6 μg/ml as compared to 17.0 μg/ml in healthy controls ($P = 0.0098$). The Ficolin-1 plasma concentration was negatively correlated with SLE Disease Activity Index (SLEDAI) ($Rho = -0.29$, $P = 0.015$) and positively correlated to the [Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index] (SDI) ($Rho = 0.27$, $P = 0.026$). The Ficolin-1 concentration was also associated with the occurrence of arterial ($P = 0.0053$) but not venous thrombosis ($P = 0.42$). Finally, deposition of C4, C3 and TCC in the Ficolin-3 pathway were all correlated to SLEDAI, respectively ($P < 0.0076$).

The Ficolin-1 association to SLEDAI and SDI as well as arterial thrombosis shown in this study suggests that Ficolin-1 may be a potential new biomarker for patients with SLE. Furthermore, Ficolin-3 mediated complement activation may be valuable in monitoring disease activity in SLE patients due to the high sensitivity for complement consumption in the assay independent of the Ficolin-3 concentration.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a defined yet unsolved pathogenesis potentially affecting multiple organ systems. The course of the disease is unpredictable and involves flares with high disease activity and periods of remission. Furthermore, SLE is characterized by diffuse and individual clinical manifestations making it complicated to treat. Standard biomarkers for inflammatory diseases such as C-reactive protein cannot be used in SLE (Gaitonde et al., 2008) and thus new candidates are needed to improve diagnosis, prognosis and treatment.

As part of innate immunity, the complement system is activated through three rather distinct pathways. The classical pathway is initiated by the binding of the pattern recognition molecule C1q to antibody-antigen complexes. The alternative pathway is continuously being activated through slow, spontaneous hydrolysis of the central complement molecule C3. Finally, the lectin pathway can be activated by five different pattern recognition molecules: mannose binding lectin (MBL), Collectin-11 (CL-K1), Ficolin-1 (M-ficolin), Ficolin-2 (L-ficolin) and Ficolin-3 (H-ficolin or Hakata antigen) in association with the MBL/Ficolin associated serine proteases (MASP's) (Ma et al., 2013b; Thiel, 2007). Upon the initial activation, the complement cascade leads to massive deposition of complement fragments C4, C3 and formation of the terminal complement complex (TCC) resulting in opsonisation, chemotaxis and direct cell lysis. (Ricklin et al., 2010; Walport, 2001).

Besides acting as a fundamental part of the first line of defence against invading pathogens, the complement system is a vital

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component in tissue homeostasis. C1q was the first of the recognition molecules shown to bind specifically to apoptotic cells and mediate their clearance. Genetic deficiency of C1q and impairment of this mechanism is strongly associated to the occurrence of SLE (Botto et al., 1998; Korb and Ahearn, 1997; Mevorach et al., 1998). Later, also the initiator molecules of the lectin pathway i.e. MBL (Nauta et al., 2003; Ogden et al., 2001) and the ficolins have been shown to bind to apoptotic and necrotic cells (Honoré et al., 2007; Jensen et al., 2007; Ma et al., 2013a).

The ficolins are multimeric recognition molecules with high sequence similarities and binding specificity for especially acetylated ligands (Garlatti et al., 2010). Ficolin-1 is atypical compared to Ficolin-2 and -3 since it is expressed primarily in monocytes and granulocytes from where the molecules are secreted. Once released it binds back to the surface of the monocytes and granulocytes in a calcium-dependent manner (Honoré et al., 2010; Rørvig et al., 2009) and is consequently found in a relatively low mean concentration of 0.3 μg/ml in serum under normal conditions (Munthe-Fog et al., 2012). Ficolin-2 is expressed in the liver and circulates the blood stream in a mean concentration of 5 μg/ml (Munthe-Fog et al., 2007). Ficolin-3 was first discovered as a thermolabile β_2-macroglycoprotein and as an autoantigen in SLE patients (Inaba et al., 1990; Sugimoto et al., 1998). Of the recognition molecules in the lectin pathway Ficolin-3 circulates with the highest concentration in serum with a mean of 25 μg/ml (Munthe-Fog et al., 2008) and of the ficolins it is the most potent activator of the complement cascade (Hummelshoj et al., 2008).

In the pathogenesis of SLE the complement system plays a dual role. On one hand, as mentioned above, genetic deficiency of complement factors is associated with the occurrence of SLE. On the other hand, massive complement activation in the period of flares is a major contributor to tissue inflammation in SLE. Recently, we added an assay for assessing the Ficolin-3 mediated lectin pathway to the complement evaluating assays (Hein et al., 2010). Thus, the aim of this study was to investigate the possible association between the ficolins and the lectin pathway and different disease manifestations in SLE.

2. Materials and methods

2.1. Materials

Maxisorp microtiter plates (439,454) were purchased from NUNC (Roskilde, Denmark). PBS-buffer (10 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4), Barbital-buffer (4 mM $C_8H_{11}N_2NaO_3$, 145 mM NaCl, 2.6 mM $CaCl_2$, 2.1 mM $MgCl_2$, pH 7.4) and 1 M sulphuric acid were all acquired from the hospital pharmacy (Rigshospitalet, Copenhagen, Denmark). CrossDown buffer (A6485) used in the Ficolin-1 assay was from Applichem (Darmstadt, Germany). Tween-20 was purchased from Merck (Hohenbrunn, Germany). Bovine serum albumin (BSA) (A3803), sodium acetate solution, acetic anhydride and sodium polyanethole sulfonate (SPS) (P2008-5G) were all purchased from Sigma-Aldrich (Brøndby, Denmark). Polyclonal rabbit-anti-ficolin-1 (HP9039) was acquired from Hycult Biotech (Uden, The Netherlands).

Table 1
Demographics and clinical characteristics of SLE patients at inclusion time.

Number of patients, *n*	68
Female, *n* (%)	62 (91.2)
Male, *n* (%)	6 (8.8)
Age, mean (range)	40.1 (21–76)
Disease duration at inclusion time, mean years (range)	10.7 (0–37)
Disease index	
SLEDAI, mean (range)	5.2 (0–21)
SDI, mean (range)	0.8 (0–8)
Disease manifestations at inclusion time	
Nephritis, now, *n* (%)	13 (19.1)
Arterial thrombosis, ever, *n* (%)	15 (22.1)
Venous thrombosis, ever, *n* (%)	11 (16.2)
Vasculitis, ever, *n* (%)	19 (28.4)
Arthritis, now, *n* (%)	7 (10.3)
APS, ever, *n* (%)	17 (26.6)
Skin rash, now, *n* (%)	3 (4.4)
Sjögren's, now, *n* (%)	5 (8.1)

SLEDAI: SLE Disease Activity Index. SDI: Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index. ASP: anti-phospolipide syndrome.

Polyclonal rabbit-α-C4c antibody (Q0369), Horseradish-peroxidase (HRP) conjugated rabbit-anti-mouse IgG and OPD substrate tablets were purchased from Dako (Glostrup, Denmark). Rabbit-anti-C3c polyclonal antibody was from Dade-Behring (Marburg, Germany). Mouse-anti-human-C5b-C9 terminal complement complex (TCC) monoclonal antibody (DIA 011-01) was purchased from Bioporto Diagnostics (Gentofte, Denmark). Donkey-anti-rabbit-HRP and streptavidin-HRP were purchased from GE Healthcare (Buckinghamshire, UK).

2.2. Patients

68 Danish SLE patients fulfilling the updated American College of Rheumatology criteria for SLE (Hochberg, 1997) were included in the study. The patient cohort and control individuals have previously been described in detail (Nielsen et al., 2011); patient demographics are summarized in Table 1. In brief, disease activity was scored according to the Safety of Estrogen in Lupus Erythematosus International Assessment version (Petri et al., 2005) of SLE Disease Activity Index (SLEDAI) describing the current activity of the disease in the patient (Bombardier et al., 1992). Chronic, irreversible organ damage was recorded using the Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index (SDI) (Gladman et al., 1996; Stoll et al., 1996). Clinical manifestations were assessed in accordance with SLEDAI. In Table 1, arterial and venous thrombosis, vasculitis and anti-phospholipid syndrome (APS) includes patients with a history of any incidence of this manifestation ever; whereas nephritis, arthritis, skin rash and Sjögrens refer to patients with active manifestation at inclusion time. 46 patients were receiving disease modifying anti-rheumatic medication (DMARD) at inclusion time. 29 healthy individuals consisting of 24 women and 5 men with a median age of 37 years (range 22-71) were included

Table 2
Spearman rank correlation between disease scores and ficolins or Ficolin-3 mediated complement deposition.

	Concentration						Ficolin-3 complement deposition					
	Ficolin-1		Ficolin-2		Ficolin-3		C4		C3		TCC	
	rho	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*
SLEDAI	−0.29	0.015	−0.25	0.052	0.05	0.66	−0.35	0.0039	−0.39	0.0024	−0.36	0.0026
SDI	0.27	0.026	−0.18	0.36	−0.06	0.66	0.03	0.83	−0.02	0.88	0.16	0.20

SLEDAI: SLE Disease Activity Index. SDI: Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index.