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Anti-C1q autoantibodies from systemic lupus erythematosus patients activate the complement system via both the classical and lectin pathways☆

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ABSTRACT

Autoantibodies against complement C1q (anti-C1q) strongly correlate with the occurrence of lupus nephritis and hypocomplementemia in systemic lupus erythematosus (SLE). Although a direct pathogenic role of anti-C1q has been suggested, the assumed complement-activating capacity remains to be elucidated. Using an ELISA-based assay, we found that anti-C1q activate the classical (CP) and lectin pathways (LP) depending on the anti-C1q immunoglobulin-class repertoire present in the patient's serum. IgG anti-C1q resulted in the activation of the CP as reflected by C4b deposition in the presence of purified C1 and C4 in a dose-dependent manner. The extent of C4b deposition correlated with anti-C1q levels in SLE patients but not in healthy controls. Our data indicate that SLE patient-derived anti-C1q can activate the CP and the LP but not the alternative pathway of complement. These findings are of importance for the understanding of the role of anti-C1q in SLE suggesting a direct link to hypocomplementemia.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by B cell hyperactivity, antibodies specific for various auto-antigens, the formation of immune complexes (ICs), the aberrant activation of the complement system resulting in complement deposition and hypocomplementemia [1,2].

The complement system is an integral part of the innate immune system, consisting of several plasma and cell-associated proteins, and acting upon triggering as a cascade resulting in the opsonization and lysis of targeted cells (e.g., pathogens), in the production of anaphylatoxins (C3a, C5a), and in the recruitment of immune cells to the site of local inflammation [3]. The complement system can be activated by at least 3 different pathways: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP) [3,4]. Each of these pathways is initiated by a specific ligand. The CP is activated by binding of the C1 complex to ICs, the LP by binding of mannose-binding lectin (MBL) or ficolins to e.g., mannose-containing groups on bacterial surfaces, and the AP by spontaneous C3(H_2O) binding and activation/turn-over or by bacterial products (e.g., lipopolysaccharide (LPS)) [3,4]. All 3 pathways converge in the formation of the C3 convertase, the cleavage of C3 into C3a and C3b, further downstream activation of terminal complement components and finally in the assembly of the membrane-attack complex (C5b–C9) [5].

Primary deficiencies of early components of the CP (C1q, C4, C2) are strongly associated with SLE. In particular, homozygous C1q deficiency was shown to be the strongest genetic susceptibility to develop SLE [6,7]. This observation suggests that complement plays a major role in the pathogenesis of SLE. However, most SLE patients do not suffer from primary C1q deficiency, but aberrant complement activation is accounted for low complement levels [8]. A rational reason for low C1q levels are autoantibodies against C1q (anti-C1q) that are present in 20–50% of unselected SLE patients. Anti-C1q levels in these patients not only correlate with hypocomplementemia but also with the occurrence of proliferative lupus nephritis [9–11]. Limited evidence is available supporting a direct role of these autoantibodies in the pathogenesis of lupus nephritis. Animal models suggest that renal inflammation is not induced by anti-C1q deposited in the kidneys together with

Abbreviations: AB, antibody; anti-C1q, anti-C1q autoantibodies; AP, alternative pathway; AU, arbitrary units; CP, classical pathway; C1qDS, C1q-depleted serum; HS-PBS, high-salt PBS; ICs, immune complexes; Ig-class, immunoglobulin-class; imC1q, immobilized C1q; IQM, interquartile mean; LP, lectin pathway; LPS, lipopolysaccharide; MBL, mannose-binding lectin; NHS, normal human sera; PBS, phosphate buffered saline; RA, rheumatoid arthritis; RT, room temperature; SLE, systemic lupus erythematosus; VBS, veronal buffered saline.

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C1q alone. Anti-C1q were only found to be pathogenic in these models in combination with preformed glomerular C1q-containing ICs [12]. Anti-C1q were shown to bind to a cryptic epitope only exposed when C1q is surface-bound, but that is shielded on C1q in fluid-phase [13]. Importantly, anti-C1q specifically target C1q bound to early apoptotic cells [14,15], providing a link between SLE, C1q, and apoptosis.

Little is known about the complement-activating potential of anti-C1q bound to immobilized C1q (imC1q) or which activation pathways might be triggered by anti-C1q. In a recent study, Pang et al. demonstrated that affinity purified anti-C1q of lupus nephritis patients inhibited the activation of the CP using ICs as the initiating structure [15]. In contrast, other studies showed that autoantibodies in general have rather complement-activating capacities [16]. However, the complement pathway that is primarily triggered by autoantibodies seems not only to be dependent on the dominant immunoglobulin-class (Ig-class) but also on the target antigen. In an in vitro assay, anti-citrullinated protein antibodies from rheumatoid arthritis (RA) patients were found to activate both, the CP as well as the AP of complement [17], whereas anti-neutrophil cytoplasmic autoantibodies were described to primarily activate the AP without apparent signs of LP or CP activation [18]. Others demonstrated that ICs consisting of collagen and anti-collagen autoantibodies could activate both the CP and the AP [19]. Also, anti-phospholipid antibodies have been linked to complement activation whereby the CP was found to be the initiator and the AP the amplifier of complement activation [20]. Finally, cryoglobulins were shown to activate all 3 pathways of complement, the CP, the LP, and the AP [21].

The aim of this study was to elucidate the complement-activating potential of SLE patient-derived high-affinity anti-C1q. Comprehension of the complement-activating potential of anti-C1q will improve the understanding of the disease and support the development of complement-targeting treatments.

2. Patients and methods

2.1. Anti-C1q source

A cohort of 27 SLE patients (Table 1) and 25 healthy control donors were included in the study. All SLE patients fulfilled at least 4/11 criteria of the American College of Rheumatology [22,23]. Collection and use of serum samples were approved by the local Ethics Committee (EKZ-No.: 110/04; 130/05). Before serum samples were diluted to their final working concentration, each sample was heat-inactivated (56 °C, 30 min) and centrifuged at 14,000 ×*g* for 30 min at 4 °C.

2.2. Complement-active/-deficient sera and dilution buffers

As a source of complement, normal human sera (NHS) from healthy donors were aliquoted and stored at −80 °C until used. These NHS had normal C1q and MBL levels and no detectable anti-C1q levels. In addition, we used MBL-deficient sera from healthy donors (undetectable MBL; but normal C1q levels) and C1q-depleted serum (C1qDS; Complement Technology: negative for C1q; but normal MBL levels).

Table 1
SLE patient characteristics.

Sex (females/males)	22/5
Age (mean (range), years)	47 (28–71)
Hypocomplementemia (low C3 and C4) (yes/no)[a]	14/13
Lupus nephritis (yes/no)[a]	16/11
Positive for anti-C1q[a]:	20
– Positive for IgG anti-C1q	9
– Positive for IgG and IgM anti-C1q	3
– Positive for IgG and IgA anti-C1q	5
– Positive for IgG, IgM, and IgA anti-C1q	3
Negative for anti-C1q[a]	7

[a] Information at time point of blood sampling.

Purified complement proteins and sera used as a source of complement were diluted either using veronal buffered saline (VBS: 5 mM barbituric acid/0.5 mM $MgCl_2$/2 mM $CaCl_2$/140 mM NaCl/0.05% Tween, pH 7.5), Mg–EGTA buffer (modified VBS: 10 mM EGTA/15 mM $MgCl_2$/140 mM NaCl/0.05% Tween, pH 7.5), or PBS–EDTA (PBS/10 mM EDTA/0.05% Tween, pH 7.5). The presence of 0.05% Tween did not impair the activation of any of the complement pathways (data not shown).

2.3. Detection of anti-C1q levels

For the detection of anti-C1q levels, ELISA plates (MaxiSorp, Nalge Nunc International) were coated with purified C1q (5 μg/ml; Complement Technology) in coating buffer (0.1 M sodium carbonate buffer, pH 9.6) overnight at 4 °C. After each incubation step, the plates were washed 3 times with washing buffer (PBS-T: PBS/0.05% Tween 20). C1q-coated plates were incubated with serum samples from SLE patients or healthy donors diluted 1:50 in high-salt buffer (HS-PBS: PBS-T/1 M NaCl) for 1 h at 37 °C. After washing, different anti-C1q classes (IgG, IgA, IgM) were detected with specific antibodies diluted in HS-PBS for 1 h at room temperature (RT). Bound IgG was detected with alkaline phosphatase (AP)-conjugated rabbit anti-IgG (Promega), bound IgA with AP-conjugated goat anti-IgA, and bound IgM with AP-conjugated donkey anti-IgM (both obtained from Jackson ImmunoResearch). The enzyme activity of AP was detected by incubating plates with AP substrate (Sigma-Aldrich) according to the manufacturer's instructions. Absorbance was read at 405 nm using a microplate biokinetics reader (BioTek Instruments). For data analysis the results were standardized as follows: measurements were expressed in units relative to the O.D. values of a reference SLE serum having high anti-C1q levels and corresponding to 1000 arbitrary units (AU). The reference serum was used as an internal control and included on each plate in each experiment. The cut-off was determined as the interquartile mean (IQM) of the AU obtained with NHS plus 3 times the SD. Every sample was tested in duplicate within a single experiment, and experiments were performed 3 times.

2.4. Complement activation assays

2.4.1. Detection of CP, LP, and AP activation

Functional activity of the 3 complement activation pathways was analyzed by ELISA using coated IgM (2 μg/ml) for the CP, coated mannan (100 μg/ml; *Saccharomyces cerevisiae*; M7504) for the LP, and coated LPS (10 μg/ml; *Escherichia coli*: 0127:B8, all obtained from Sigma-Aldrich) for the AP. Plates were coated over night at 4 °C. Residual binding sites were blocked with blocking buffer (BSA–PBS: PBS/1% BSA) for 1 h at RT. Then, the plates were washed and incubated for 1 h at 37 °C with NHS diluted at 1% (for the CP and the LP) or 10% (for the AP) in VBS, Mg–EGTA buffer, or PBS–EDTA buffer as a source of complement. After a wash step, deposited C3 was detected by goat anti-C3 (Quidel) and deposited C4b by goat anti-C4b (Complement Technology). For both a secondary HRP-labeled mouse anti-goat IgG (Sigma-Aldrich) was used. After a final washing step, HRP activity was quantified by using TMB substrate (BD Biosciences) according to the manufacturer's instructions. The reaction was stopped by the addition of 4 M H_2SO_4 and absorbance was read at 450 nm.

2.4.2. Complement activation by anti-C1q

To investigate the ability of anti-C1q in activating the complement system, ELISA plates were coated with C1q in coating buffer overnight at 4 °C and free binding sites were blocked with PBS–BSA for 1 h at RT. After a washing step, plates were incubated for 10 min at 37 °C with heat-inactivated sera from SLE patients or healthy donors diluted in HS-PBS. This short 10 min incubation time was found to be sufficient for anti-C1q binding to imC1q in preliminary kinetic experiments (data not shown). Then, plates were washed again. In case of using sera as a source of complement for the assay, plates were incubated