Anti-C1q Autoantibodies in Lupus Nephritis

Prevalence and Clinical Significance

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ABSTRACT: Recently, anti-C1q autoantibodies have been proposed as a useful marker in systemic lupus erythematosus (SLE) since their occurrence correlates with renal involvement and, possibly, with nephritic activity. We aimed to evaluate the prevalence of anti-C1q antibodies in patients with SLE, with and without renal involvement, and to correlate these markers' presence and levels with the activity of the disease and nephropathy. We studied 61 patients with SLE, 40 of whom had biopsy-proven lupus nephritis; 35 patients with other connective tissue diseases; and 54 healthy controls. In addition, 18 lupus nephritis patients were followed up during the disease time course. Anti-C1q antibodies were measured using "homemade" ELISA with high salt concentration (1 M sodium chloride). High anti-C1q antibody titers (> 55 AU) were present in 27 of 61 (44%) SLE patients and in 4% and 0% of normal blood donors and pathologic controls, respectively. Anti-C1q antibodies were found in 60% of patients with lupus nephritis compared with only 14% of SLE patients without nephropathy ($P < 0.05$). Moreover, patients who were positive for anti-C1q antibodies had a higher European Consensus Lupus Activity Measurement (ECLAM) score (4.35 vs. 2.2); 89% of patients with active lupus nephritis showed high titers of anti-C1q antibodies compared with 0% of patients with inactive nephritis. Anti-C1q and anti-dsDNA antibodies agreed in 79% of cases. Our results confirm that anti-C1q antibodies are present in a significant percentage of SLE patients, and that their presence and levels correlate with disease activity—in particular, during renal flare-ups.

KEYWORDS: systemic lupus erythematosus; SLE; glomerulonephritis; renal flares; anti-C1q antibodies; anti-DNA antibodies; lupus nephritis; autoimmune disease

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INTRODUCTION

Since 1971, it has been known that sera from patients with systemic lupus erythematosus (SLE) contain low- (7S) and high-molecular-weight material binding to C1q,[1] but it was more than fifteen years later before this low-molecular-weight type was identified as monomeric IgG with antibody activity against the collagen-like region of C1q.[2] At that time, a solid-phase assay using purified C1q, coated onto plastic tubes, was used for detecting circulating immune complexes. Using density-gradient ultracentrifugation, researchers demonstrated that not only immune complexes, but also monomeric IgG, bound to solid-phase C1q. Subsequently, an enzyme-linked immunosorbent assay (ELISA) method for detecting anti-C1q antibodies was developed. Using high salt concentrations (0.5–1.0 M sodium chloride), it became possible to differentiate between immune complexes and anti-C1q antibodies (since, under these conditions, the binding of the globular heads of C1q to immune complexes was prevented).[3]

Using this test, researchers discovered that, in addition to SLE, anti-C1q antibodies are found in a variety of diseases, and even in some apparently normal individuals (reviewed by Seelen *et al.*[4]). In particular, anti-C1q antibodies are detected at a high titer in 100% of patients with hypocomplementemic urticarial vasculitis syndrome.[5]

Anti-C1q antibodies are found in the bloodstream of 30–48% of SLE patients, and their presence is associated with renal involvement.[4] More important, anti-C1q antibodies have been correlated with active renal disease with a sensitivity of 44–100% and a specificity of 70–92%.[6,7] Note that this strong association has been found pertaining only to renal involvement in lupus patients.[3,8] Moreover, researchers have suggested that an increase in anti-C1q antibody titer can predict renal flare-ups in lupus nephritis.[9,10]

The prognostic value (as markers of renal disease activity) of other, more widely used, serological tests (such as anti-double-stranded DNA [anti-dsDNA] antibodies and complement levels) is quite controversial.[11] Thus, the aims of our study were as follows:

(1) To evaluate the prevalence of anti-C1q autoantibodies in SLE patients and control groups;
(2) To verify the correlation with renal involvement and renal disease activity; and
(3) To compare the performance of anti-C1q antibodies for predicting renal flare ups with that of other commonly used assays (anti-dsDNA antibodies and complement levels).

PATIENTS AND METHODS

Patients

We studied 99 serum samples from 40 patients with biopsy-proven lupus nephritis (34 with proliferative forms, WHO Class III or IV; and 6 with membranous nephropathy, WHO Class V) and 35 serum samples from 21 lupus patients without renal

involvement. Patients were followed at the Department of Nephrology and Immunology of the Ospedale San Carlo Borromeo in Milano, Italy. All patients fulfilled the 1982 revised American College of Rheumatology (ACR) criteria for SLE.[12] The control groups consisted of 89 people: 35 patients with non-SLE connective tissue diseases (i.e., progressive systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease [MCTD], undifferentiated connective tissue disease, antiphospholipid syndrome) and 54 blood donors.

Serum samples were collected during either a quiescent phase or an active phase of renal disease. The quiescent phase was defined as stable creatinine clearance for at least 6 months, proteinuria of less than 0.5 g/day of protein, and inactive urinary sediment (in the absence of extrarenal signs).[6] Renal flare-ups were defined as (*a*) nephritic flare-ups, characterized by an increase in plasma creatinine level of at least 30% greater than the last value, associated with nephritic urinary sediment; and (*b*) proteinuric flare-ups, characterized by either stable plasma creatinine levels with an increase in proteinuria of at least 2 g/day of protein (if basal proteinuria was less than 3.5 g/day), or doubled proteinuria if the patient already had nephrotic proteinuria.[6] Overall SLE disease activity was measured by means of the European Consensus Lupus Activity Measurement (ECLAM).[13]

Methods

Anti-dsDNA antibodies were measured using the Farr assay (Amersham Biosciences, Buckinghamshire, UK [now part of GE Healthcare]); C3 and C4 plasma levels were measured by nephelometry.

Anti-C1q antibodies were detected using the method described by Siegert and colleagues,[14] with minor modifications. Briefly, 96-well microtiter plates (Greiner Bio-One) were coated overnight with a 10 μg/mL of human C1q in phosphate-buffered saline (PBS) at 4°C. After washing with PBS-Tween 20, plates were blocked with PBS containing 2% fetal calf serum (FCS) for 1 hour at 37°C. After washing, the serum samples (diluted 1/200 in PBS-Tween 20 containing FCS 2% and 1.0 molar/L NaCl) were added to the wells and incubated for 2 hours at room temperature. Serum samples had been previously centrifuged at 16,000*g* for 5 minutes to remove aggregates. Bound IgG was detected using an alkaline phosphatase conjugated F(ab)2 goat anti-human IgG (Sigma) followed by the proper substrate. The results were expressed as arbitrary units (AUs) by reading off a standard curve composed of a pool of positive sera. The normal range was calculated using receiver operating characteristic (ROC) curves.

Statistical Analysis

All the analyses were performed using SPSS software (Cary, NC). The differences between anti-C1q-positive and anti-C1q-negative patients in terrms of continuous variables were tested by the Mann-Whitney U-test, and in categorical variables by Fisher's exact test. Spearman rank and Pearson's correlation coefficient were used to test the correlation between continuous variables (or their log transformation, when necessary). All reported *P* values are two-sided. A *P* value of less than 0.05 was regarded as statistically significant.

TABLE 1. Anti-C1q antibodies in SLE patients and control groups: titer and percentage testing positive

	SLE ($n = 21$)	SLE-GN ($n = 40$)	CTD ($n = 35$)	NHC ($n = 54$)
Mean (SD)	37 (55)	134 (118)[a]	11 (4)	23 (50)
Positive (%)	14	60[a]	0	4

ABBREVIATIONS: CTD, connective tissue disease; GN, glomerulonephritis; NHC, normal healthy control; SD, standard deviation; SLE, systemic lupus erythematosus. Normal values: <55 AU (arbitrary units).
[a]$P < 0.05$.

TABLE 2. Correlations between the presence of anti-C1q antibodies and other clinical and laboratory parameters

	R	*P* value
Anti-dsDNA	0.68	0.0001
ECLAM	0.57	0.0001
C3 plasma level	–0.44	0.0001
C4 plasma level	–0.43	0.0001
Hematuria	0.38	0.0002
ESR	0.26	0.0002
Proteinuria	0.23	0.0002

ABBREVIATIONS: dsDNA, double-stranded DNA; ECLAM, European Consensus Lupus Activity Measurement; ESR, erythrocyte sedimentation rate.

RESULTS

High anti-C1q antibody titers (>55 AU) were present in 27/61 (44%) of SLE patients and in 4% and 0% of normal blood donors and pathological controls, respectively. TABLE 1 summarizes the results of anti-C1q antibodies both in SLE patients, with and without lupus nephritis, and in control groups. Patients with lupus nephritis had a statistically significant higher titer of anti-C1q antibodies compared with both normal controls, patients with non-SLE connective tissue diseases, and SLE patients without renal involvement. Sixty percent of lupus nephritis patients tested positive for anti-C1q antibodies compared with 14% of SLE patients without nephritis ($P < 0.05$).

Serum levels of anti-C1q antibodies showed a positive correlation with levels of anti-dsDNA antibodies, ECLAM score, magnitude of hematuria and proteinuria, and erythrocyte sedimentation rate (ESR); they showed a negative correlation with plasma levels of C3 and C4 (TABLE 2). Other significant results include the following:

- Patients who tested positive for anti-C1q antibodies had higher ECLAM scores compared with SLE patients without anti-C1q antibodies (4.35 vs. 2.2).

TABLE 3. Correlations between renal flares and immunologic parameters in SLE

	P value
Anti-C1q	0.0001
Anti-dsDNA	0.0003
C3 plasma level	0.0004
C4 plasma level	0.0030

- Of patients with active lupus nephritis, 89% showed high titers of anti-C1q antibodies compared with 0% of patients with inactive nephritis.
- Anti-C1q and anti-dsDNA antibodies agreed in 79% of cases.
- When the presence or absence of renal flare-ups was correlated with immunologic parameters, the best correlation was found with anti-C1q antibodies, followed by anti-dsDNA and complement levels (TABLE 3). The sensitivity/specificity of anti-C1q antibodies for active renal disease were 86% and 95%, respectively, compared with 79% and 84% for anti-dsDNA antibodies. Both assays combined had a sensitivity of 91% and a specificity of 90%.

DISCUSSION

The present study has confirmed the presence of anti-C1q antibodies in a high percentage of SLE patients. Anti-C1q antibodies were found in 44% of our lupus patients, a number comparable with that reported in other studies.[15–18] We found a strong association between anti-C1q antibodies and (active) renal disease. Even though in our patients anti-C1q antibodies were shown to correlate with other immunologic parameters of disease activity (e.g., complement reduction and anti-dsDNA antibodies), renal flare-ups were related to C1q antibodies more strongly than to these assays.

SLE is characterized by the presence of a wide variety of autoantibodies, comprising antinuclear, anti-Sm, antiphospholipid, and anti-dsDNA antibodies, which are included in the ACR classification criteria and are therefore considered helpful for diagnosis.[12] Anti-dsDNA antibodies and complement levels are also useful for monitoring disease activity. However, even though high levels of anti-dsDNA antibodies are related to lupus nephritis, increases in anti-dsDNA antibodies cannot clearly distinguish between extrarenal and renal relapses.[4,6,10] Moreover, these antibodies are also found in a relatively high percentage in both clinically inactive disease and patients without nephritis.

Glomerulonephritis is a frequent and often severe feature of SLE and is one of the major determinants of poor outcome. Reliable markers for diagnosing and monitoring lupus nephritis are therefore critically important.

With few exceptions,[15] anti-C1q antibodies have been clearly associated with active renal disease in SLE, and especially with severe, proliferative forms.[4,6,8–10,16,17]

It has been postulated that no lupus nephritis can occur in the absence of anti-C1q antibodies.[7,18] Indeed, in our series as well, all the patients with severe proliferative glomerulonephritis had high levels of anti-C1q antibodies, which became undetectable during treatment-induced remission. Whereas anti-dsDNA antibody titer does not seem to be able to accurately predict renal exacerbations, in our experience (as in that of others[6,8,10]), the sensitivity/specificity of anti-C1q antibodies for renal disease activity and renal flares were found to be greater than that of anti-dsDNA antibodies or C3/C4 plasma levels.[6,10]

The association of anti-C1q antibodies with lupus nephritis has been demonstrated not only by the clinical correlation of anti-C1q antibody positivity with active nephritis, but also by the discovery of anti-C1q antibodies in lupus nephritis kidneys.[19,20] Although anti-C1q antibodies are found in a number of diseases other than SLE, and in up to 4% of apparently healthy individuals (reviewed in Seelen[4]), most of these diseases (e.g., hypocomplementemic urticarial vasculitis syndrome, Felty's syndrome, rheumatoid arthritis, rheumatoid vasculitis, classic polyarteritis nodosa, MCTD, and Sjögren's syndrome) are not usually characterized by renal involvement and, in particular, by proliferative glomerulonephritis.

However, do anti-C1q antibodies contribute to the pathogenesis of lupus nephritis, or do they represent only a useful serological marker? Recently, new clues have emerged to explain such controversial findings. For example, it has been shown in experimental models that administering a mouse anti-mouse C1q monoclonal antibody to treatment naive mice resulted in glomerular deposition of C1q and anti-C1q autoantibodies but not in overt renal disease. However, administering anti-C1q antibodies to mice pretreated with C1q-fixing antiglomerular basement membrane antibodies (a model for glomerular immune complex disease) resulted in strong synergistic enhancement of renal disease.[21] Thus, it appears that anti-C1q autoantibodies can be pathogenic to the kidney, but only in the context of C1q-containing glomerular immune complexes, as found in SLE.[21] Moreover, these autoantibodies could serve as an acquired mechanism of complement classical pathway amplification.[22]

Not only is C1q important for complement activation, but it can also help to clear away potentially dangerous nuclear autoantigens from apoptotic cells. Thus, the absence of C1q leads to the development of anti-DNA antibodies and to clinical SLE.[22] In the context of multiple roles for C1q, researchers have hypothesized that anti-C1q autoantibodies affect patients with SLE not only by injuring the kidneys, but also by enhancing the development of anti-DNA and other glomerular-targeting nuclear autoantibodies, because there is too little C1q available for effective clearance of these dangerous antigens.[22] Indeed, researchers have demonstrated an inverse correlation between anti-C1q autoantibody titers and plasma C1q levels.[17] Thus, these autoantibodies play a dual role: not only can they amplify local injury, but they can also accelerate the development of antinuclear autoantibodies by interfering with C1q clearance functions.[22]

What causes anti-C1q reactivity to develop is still unknown. Seelen and colleagues have suggested that in SLE, anti-C1q reactivity might be caused by the large-scale exposure of C1q neo-epitopes during massive complement activation throughout the active course of the disease.[4] Alternatively, impaired and aberrant clearance of apoptotic material might give rise to autoantibody formation to antigens present on apoptotic material, such as C1q.[6]

CONCLUSIONS

Anti-C1q autoantibodies are present in a high percentage of patients with lupus nephritis. These antibodies correlate with renal disease activity and with renal flare-ups better than do other immunologic parameters, such as anti-dsDNA and complement levels. Although by themselves anti-C1q antibodies are not pathogenic, they can exacerbate renal disease when C1q-containing immune complexes are present in the glomerulus. Furthermore, they can increase the severity of a person's autoimmune response by interfering with C1q's functions of clearing out apoptotic material. Thus, anti-C1q antibodies can represent a useful tool for monitoring disease course, prognosis, and response to treatment in SLE patients.

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