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Anti-C1q Antibodies: Association With Nephritis and Disease Activity in Systemic Lupus Erythematosus

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Background: Anti-C1q antibodies have been described in systemic lupus erythematosus (SLE) as well as in other connective tissue diseases. They have been considered as a marker for disease activity and presence of nephritis.

Objective: The aim of this study was to determine the prevalence of anti-C1q antibodies in Brazilian lupus patients as well as analyze their association with different clinical and serologic parameters.

Methods: Sera from 81 SLE patients, based on the American College of Rheumatology (ACR) criteria, were collected from a lupus referral outpatient clinic in Salvador, Brazil. Antibodies to C1q were detected by an enzyme-linked immunoassay (ELISA) kit and antibodies to other cellular antigens identified by indirect immunofluorescence on HEp-2 cell substrate (ANA), or *Crithidia luciliae* (dsDNA), and to nucleosome by ELISA. A cutoff of 20 U was established for anti-C1q and antinucleosome assays.

Results: Anti-C1q antibodies were detected in 39.5% (32/81) of SLE sera. The presence of anti-C1q antibodies was associated with proteinuria ($P = 0.028$) but not with other laboratory or clinical features, such as antinucleosome or anti-dsDNA antibodies, hematuria, urinary casts or renal failure, leukopenia, pericarditis, pleuritis, malar rash, seizures, and psychosis. There was a positive correlation between the titers of anti-C1q antibodies and the systemic lupuis erythematosus disease activity index (SLEDAI) score ($r = 0.370$; $P = 0.001$).

Conclusion: This study in Brazilian SLE patients confirms previous findings of the association of anti-C1q antibodies with nephritis and disease activity. J. Clin. Lab. Anal. 23 : 19–23, 2009.

Key words: autoantibodies; anti-C1q antibodies; systemic lupus erythematosus; nephritis

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic disorder involving several autoantibodies with a potential etiopathogenic role, such as anti-dsDNA, antiphospholipid, and anti-SSA (Ro). Likewise, impairment of clearance of immune complexes and apoptotic cells has been considered as an important factor contributing to the development of the disease. Moreover, deficiency of complement components has also been classically associated with the development of SLE. One case of this type is homozygous C1q deficiency (1).

More recently, several studies have demonstrated the presence of antibodies directed to this component of

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complement in SLE (2–5), as well as in other connective tissue diseases (6,7) and hepatitis C infection (7). Nevertheless, in SLE their presence has been associated with disease activity, particularly renal flares of lupus nephritis (5,8,9). The role of C1q in clearance of apoptotic cells and immune complexes is the probable mechanism linking its deficiency or inhibition with autoimmune diseases (10,11).

The aim of this study was to investigate the frequency of anti-C1q antibodies in a group of Brazilian SLE patients and evaluate their association with different clinical features of the disease.

MATERIALS AND METHODS

Patient Enrollment

Unselected SLE patients were collected from a lupus referral outpatient clinic in Salvador, Brazil. The diagnosis of SLE was based on the ACR criteria (12). Disease activity was evaluated by SLEDAI (13). Patients infected with Virus C hepatitis were not included in the study.

Proteinuria was defined as 500 mg/24 hr or higher in the last 10 days, renal dysfunction as any increase in creatinine value at any time in the history, and renal involvement as any of the two above variables. Other clinical features were labeled if present at any time in the course of the disease.

The study had the approval of the Ethics Review Board of our institution and each patient signed a consent form on enrollment.

Laboratory Procedures

Aliquots of sera were stored at $-20^{\circ}C$ until needed and all autoantibody testing was performed at the Laboratory of Molecular Biology, Gonçalo Muniz Foundation, Bahia, Brazil. This included an autoantibody analysis by indirect immunofluorescence on HEp-2 substrate (ANA) and *Crithidia luciliae* (anti-dsDNA). Antibodies to nucleosome were measured by enzyme-linked immunoassay (ELISA) (INOVA Diagnostics Inc., San Diego, CA).

Anti-C1q antibodies by ELISA: An ELISA kit was used to detect anti-C1q as follows. Patient samples were diluted 1/100 and then added to each well. After a 30-min incubation, the wells were washed with high-ionic-strength buffer to remove immunoglobulins bound to the globular head region of C1q, leaving behind specific anti-C1q antibodies. Then horseradish peroxidase coupled to anti-human IgG conjugate supplied with the kit was used as the secondary antibody. After developing the reaction with the chromogen supplied, the absorbance was read at 450 nm. Values <20 U were considered as negative, and positives were classified as "weak" (20–39 U), "moderate" (40–80 U), and "strong" (>80 U) as suggested by the manufacturer.

Statistical Analysis

All clinical and laboratory data were entered into and extracted from SPSS for Windows (version 14.0). Some results were expressed as mean $\pm$ standard deviation. The Kolmogorov–Smirnov was utilized to test the normality of the variables. The Mann–Whitney test was used to compare means. Correlation coefficient was investigated by Spearman test. The association between qualitative variables was evaluated by Yates' χ^2 with correction or Fisher's exact test, when indicated, considering $P<0.05$ as statistically significant.

RESULTS

SLE Demographics and Main Clinical Features

The unselected SLE population studied included 80 (98.7%) women and only 1 (1.3%) man with a mean age of 34 ($\pm$11) years. The median disease duration was 49 months (range 2–384). The distribution of race was 40.7% Mulatto, 29.6% Caucasoid, and 29.6% Black (Table 1). The main clinical features of the studied population are presented in Table 2.

Clinical and Serological Correlate of Anti-C1q Antibodies

When the ELISA method was used to detect anti-C1q antibodies in the SLE sera, a prevalence of 39.5% (32/81) was observed. The comparative clinical and laboratory data for anti-C1q-positive and anti-C1q-negative patients are presented in Table 3. This correlation refers to the presence of the manifestation at the time of visit or in the preceding 10 days as defined by the SLEDAI criteria.

The presence of anti-C1q antibodies was associated with proteinuria in the last 10 days, defined as higher than 500 mg/24 hr ($P = 0.028$). This association was stronger when one considers proteinuria at any time in the history ($P = 0.01$). In addition, there was a positive correlation between the titers of anti-C1q antibodies and the SLEDAI score ($r = 0.370$; $P = 0.001$) and the mean

TABLE 1. Demographic Features of 81 Systemic Lupus Erythematosus Patients

Demographic features	Results
Age (mean $\pm$ SD)	34 ($\pm$11) yr
Race (*Mulatto*/Caucasoid/Black)	40.7/29.6/29.6%
Gender (female)	80 (98.7%)
Disease duration in months (median) (range)	49 months (range 2–384)

SLEDAI score was higher in the anti-C1q-positive group ($P = 0.008$). On the other hand, there was no association with any of the clinical parameters such as arthritis, malar rash, mucosal ulcers, cutaneous vasculitis, pleuritis, urinary casts, hematuria, renal failure, fever, leukopenia, thrombocytopenia, presence of antinucleosome, anti-dsDNA antibodies, or lower levels of C3 or C4 of the complement cascade. Notably, there was no correlation between the titers of antinucleosome and anti-C1q antibodies ($r = 0.170$; $P = 0.129$).

TABLE 2. Main Clinical Features of the Studied Population of Systemic Lupus Erythematosus

Features	%
Arthritis	100
Fever	95.1
Photosensitivity	90.1
Malar rash	82.7
Mucosal ulcers	72.8
Discoid rash	16
Raynaud's phenomenon	67.9
Cutaneous vasculitis	22.2
Pericarditis	8.6
Pleuritis	14.8
Proteinuria[a]	56.8
Urinary casts	29.6
Renal dysfunction[b]	17.3
Hypertension	27.2
Psychosis	7.4
Seizures	7.4
Leukopenia[c]	64.2
Thrombocytopenia[d]	17.3
Hemolytic anemia	17.3

The percentage refers to the presence at any time in history.
[a]Proteinuria (>500 mg/24 hr).
[b]Renal dysfunction: any increase in creatinine value at any time in the history.
[c]Leukopenia: <4,000 cells/mm^3.
[d]Thrombocytopenia: <100,000 cells/mm^3.

DISCUSSION

In this study, a prevalence of 39.5% of anti-C1q antibodies was found in Brazilian SLE patients. If one takes into consideration only those with proteinuria higher than 500 mg/24 hr in the last 10 days the frequency of anti-C1q increases to 59%. This figure is similar to that observed by Sinico et al., who found anti-C1q antibodies in 27 of 61 (44%) SLE patients, and in 60% of patients with lupus nephritis, when compared with only 14% of SLE patients without nephropathy ($P < 0.05$) (5).

Curiously enough, although an association of anti-C1q and proteinuria identified in the last 10 days was found, as well as a positive correlation with SLEDAI score, no association of these antibodies was found with renal dysfunction, classified as any increase in creatinine value in the present or in the past. It may suggest that the detection of anti-C1q antibodies is a marker for disease activity, particularly in a renal site. On the other hand, the authors were unable to demonstrate any association of anti-C1q with anti-dsDNA or antinucleosome antibodies. Although similar results were observed in some studies (8,14), others found discordant results. Thus, Mosca et al. stated that anti-C1q antibodies do not seem to be related to the occurrence of flares during pregnancy (15). Braun et al. (6) found anti-C1q antibodies to be significantly correlated with anti-dsDNA. Although Oelzner et al. (14) found a positive

TABLE 3. Clinical and Serological Correlation of Anti-C1q Antibodies in 81 Systemic Lupus Erythematosus Patients[a]

Features	Entire group ($n = 81$) (%)	Anti-C1q positive ($n = 32$) (%)	Anti-C1q negative ($n = 49$) (%)	P value
Arthritis	54.3	62.5	49	0.232
Malar rash	56.8	56.3	57.1	0.937
Mucosal ulcers	30.9	31.3	30.6	0.952
Cutaneous vasculitis	8.6	6.3	10.2	0.698
Pleuritis	3.7	3.1	4.1	1.000
Proteinuria (>500 mg/24 hr)	27.2	40.6	18.4	0.028
Urinary casts	16	18.8	14.3	0.593
Hematuria	17.3	25	12.2	0.138
Fever	29.6	28.1	30.6	0.811
Leukopenia (<4,000 cells/mm^3)	54.3	50	57.1	0.528
Thrombocytopenia (<100,000 cells/mm^3)	14.8	15.6	14.3	0.868
Antinucleosome antibodies	53.1	53.1	53.1	0.996
Anti-dsDNA antibodies	38.3	40.6	36.7	0.725
C3 below normal range	22.2	31.3	16.3	0.114
C4 below normal range	37	43.8	32.7	0.312

[a]Correlation refers to the presence of the manifestation at the time of visit or in the preceding 10 d, as defined by the SLEDAI criteria. SLE, systemic lupus erythematosus.

correlation with the SLEDAI score, they did not find association with lupus nephritis.

It is still intriguing how antibodies to C1q could cause renal disease in SLE. An indirect evidence for the role of these antibodies in the pathogenesis of lupus nephritis is the observation by Chen et al. who found higher titers of anti-C1q in patients with lupus nephritis and C1q deposition in the kidney tissue (16). Other reasonable explanations would be that the autoantibodies inhibit the removal of apoptotic cells secondary to complement deficiency induced by these antibodies; they block the clearance of C1q-containing immune complexes, allowing them to deposit in the glomeruli, or they activate the complement cascade and consequently the inflammatory process (17–20).

On the other hand, the presence of anti-C1q antibodies has also been identified in the serum of patients with other connective tissue diseases without renal involvement or even in the normal population (21). Thus, the possibility that these antibodies may represent only an epiphenomenon cannot be entirely excluded. However, studies in animal models have suggested that anti-C1q may amplify complement activation, that is, when a monoclonal anti-C1q antibody alone was administered to mice, it was unable to cause renal damage, but if the level of C1q was increased in glomerulus by the previous interaction of other antibodies with glomerular antigens, the mice presented renal damage (19,20). These observations may suggest that in SLE, different from other conditions, anti-C1q antibodies have the potential to cause renal damage. The observation in this study, of no association between antinucleosome and anti-C1q antibodies, has been corroborated by others (22). In a previous study no association was found between antinucleosome and disease activity in SLE (23). Thus, these two antibodies seem to identify different subsets of SLE patients.

In conclusion, this study in Brazilian SLE patients confirms previous findings of the association of anti-C1q antibodies with nephritis and disease activity. Hence, the detection of such antibodies in SLE seems to be a useful tool for the management of these patients.

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