ASSOCIATION BETWEEN ONGOING ANTI-C1q ANTIBODY PRODUCTION IN PERIPHERAL BLOOD AND PROLIFERATIVE NEPHRITIS IN PATIENTS WITH ACTIVE SYSTEMIC LUPUS ERYTHEMATOSUS


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SUMMARY

The aim of this study was to compare ongoing production of anti-C1q antibodies (anti-C1q) in peripheral blood with serum anti-C1q levels in patients with systemic lupus erythematosus (SLE), especially in patients with nephritis. Using the ELISPOT technique for the detection of IgG and IgA anti-C1q production, 21 patients with active SLE were investigated. ELISAs for IgG and IgA anti-C1q were compared with the ELISPOT results. Six of the patients were found to have proliferative nephritis (WHO grade III/IV) confirmed by renal biopsy. High numbers of IgG anti-C1q spot-forming cells (SFC), defined as ≥20/10^5 plated peripheral blood mononuclear cells (PBMC), were exclusively observed in patients with proliferative nephritis (P<0.0001). Serum levels of IgG anti-C1q were significantly increased in patients with proliferative nephritis (P=0.039). High ongoing IgG anti-C1q production was observed in all patients with proliferative nephritis, which may be a contributory factor in the pathogenesis of this disorder. The detection of IgG anti-C1q production may be valuable in the clinical investigation of patients with suspected SLE nephritis.

KEY WORDS: SLE, Nephritis, Anti-C1q antibodies.

Systemic lupus erythematosus (SLE) is a multiorgan disease characterized by enhanced autoantibody production and immune complex formation [1]. The pathogenetic relationship between the occurrence of various autoantibodies, the presence of immune complexes and the symptoms of SLE is not fully understood. One interesting aspect in the search for such a relationship is presently emerging from reports of clinical features associated with hereditary deficiencies of early components within the classical complement cascade [2]. As these patients develop SLE or SLE-like syndromes to a very great extent, it should be of special interest to investigate the occurrence of autoantibodies to the very same complement components among SLE patients. Findings of autoantibodies to these complement components would thus indicate a pathogenetic similarity of dysfunctional complement in the two types of disorders.

The highest risk factor for the development of SLE has been reported for patients with hereditary deficiencies of C1q, the first component of the classical complement pathway [2]. In 30 patients with C1q deficiency reported in the literature, 28 developed SLE-like disorders. Further, as many as one-third of these patients developed nephritis, a prevalence comparable to that reported in idiopathic lupus.

These data raise the possibility that autoantibodies to complement components, especially C1q, may contribute to symptoms of SLE by functional interference with the target molecule, thereby causing deficient immune complex elimination (for a discussion, see [3]). Consistent with this possibility, antibodies against the collagen-like region of C1q have been reported in SLE, with an occurrence of 34–47% in mixed SLE patient populations [4, 5]. Findings of anti-C1q in serum of SLE patients have earlier been reported to be associated with nephritis, especially proliferative forms [3, 5, 6].

An obvious problem in the study of these autoantibodies is, however, the fact that anti-C1q may bind directly to C1q molecules in solid phase or in immune complexes and thereby lower the levels of anti-C1q available for measurement by serology. To overcome this constraint, we have undertaken a study to investigate ongoing antibody production of anti-C1q from peripheral blood mononuclear cells (PBMC) of active SLE patients using the ELISPOT technique. In particular, we were interested in the relationship between ongoing anti-C1q production and the occurrence of nephritis. We accordingly studied patients with active SLE for the detection of antibody production by the ELISPOT technique, which enables enumeration of numbers of anti-C1q antibody-producing cells [7, 8]. The results were then compared with the results obtained with levels of serum antibodies measured by ELISA.

Moreover, as increased anti-double-stranded (ds)DNA levels are reported to be associated with active lupus [9–11], though not exclusively with renal exacerbations, a comparison of anti-C1q and anti-dsDNA titres was included in order to evaluate
possible differences in active renal and non-renal disease.

**PATIENTS AND METHODS**

**Patients**
Twenty-one patients, all fulfilling the American College of Rheumatism criteria for SLE [12], were investigated. The patients, 19 women and two men, were selected for having an active disease flare and being subject to current investigation and/or increased therapy, and were included, if possible, before starting therapy or before receiving increased dosage of immunosuppressive treatment. Patients already treated with increased dosage of immunosuppressive agents for the actual flare were not included if the increase in dosage was initiated more than 14 days before blood sampling. This selection was performed as an earlier report had shown a rapid decline of anti-C1q production after initiation of immunosuppressive treatment, although the parallel serum levels declined much more slowly [8]. Patients receiving recent or ongoing cyclophosphamide treatment were completely excluded from the study group. For patient characteristics, see Table I.

The patients could be divided into two subsets: one group with active disease with signs of renal involvement in whom percutaneous renal biopsy was performed and one group with active non-renal disease. Renal involvement was defined by the presence of persisting proteinuria and/or the occurrence of haematuria, or by an increased serum creatinine level. Disease activity was estimated by SLE Activity Measure protocols (SLAM) [13], either prospectively or retrospectively from patient charts.

**ELISPOT technique**
At study entry, the patients were investigated for the occurrence of IgG and IgA anti-C1q spot-forming cells (SFC) and total SFC of IgG, IgA and IgM isotypes in peripheral blood by the ELISPOT technique. This technique is constructed to determine the number of cells actively producing antibodies. The analyses were performed as described earlier [8]. In brief, the cells were washed four times in phosphate-buffered saline (PBS) to avoid contamination of immunoglobulins. Fetal calf serum (free from immunoglobulins) was used in the incubation steps. The cell viability was always >98% as seen from the trypan exclusion test. Spots were enumerated with an inverted microscope, and expressed as numbers of SFC/10^5 PBMC. All results were evaluated by the same investigator (IG). As a control group for evaluation of the total SFC of IgG, IgA and IgM isotypes, 51 blood donors or healthy medical staff were used.

Duplicate wells were used for the detection of total as well as anti-C1q SFC and the results were given as mean values. No single measurement differed >15% from the mean value in the high IgG anti-C1q-producing patient group with proliferative nephritis (PN). In the whole patient group, no difference of >25% from the mean value was observed for total IgG SFC.

**TABLE I**

Characteristics of SLE patients, organ manifestations and results of ELISPOT and ELISA

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age/sex</th>
<th>Organ manifestation</th>
<th>Nephritis (WHO grade)</th>
<th>ELISPOT (SFC/10^5 PBMC)</th>
<th>ELISA C1q</th>
<th>ELISA dsDNA</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>48/F</td>
<td>R, C, MA</td>
<td>III (PN)</td>
<td>250</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>17/F</td>
<td>R, H</td>
<td>IV (PN)</td>
<td>840</td>
<td>89</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>46/F</td>
<td>R, H</td>
<td>IV (PN)</td>
<td>420</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>31/F</td>
<td>R, H</td>
<td>IV (PN)</td>
<td>2000</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>29/F</td>
<td>R, C</td>
<td>IV (PN)</td>
<td>190</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>17/F</td>
<td>R, H, S</td>
<td>IV (PN)</td>
<td>ND</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>28/F</td>
<td>R, C</td>
<td>V (n-PN)</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>39/F</td>
<td>R, H, C</td>
<td>V (n-PN)</td>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>52/F</td>
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<td>2</td>
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<tr>
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<td>V (n-PN)</td>
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<td>0</td>
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<tr>
<td>11</td>
<td>52/M</td>
<td>R, MA</td>
<td>II (n-PN)</td>
<td>40</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>65/F</td>
<td>R, H, MA</td>
<td>sclerosis</td>
<td>124</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>38/F</td>
<td>R, H, C</td>
<td>sclerosis</td>
<td>190</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>39/F</td>
<td>H, MA</td>
<td>ND</td>
<td>720</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>49/F</td>
<td>C, MA</td>
<td>ND</td>
<td>32</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>51/F</td>
<td>R (*), V, MA</td>
<td>ND</td>
<td>230</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>71/F</td>
<td>C, MA</td>
<td>ND</td>
<td>600</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>29/F</td>
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<td>ND</td>
<td>1450</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>58/F</td>
<td>H, C</td>
<td>ND</td>
<td>120</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>27/F</td>
<td>H, MA</td>
<td>ND</td>
<td>1050</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>50/M</td>
<td>CNS</td>
<td>ND</td>
<td>105</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

F, female; M, male; CNS, involvement of the central nervous system; R, clinical renal involvement; C, cutaneous manifestations; H, haematological manifestations (haemolytic anaemia, leucopenia, lymphopenia, thrombocytopenia); V, vasculitis; MA, musculoarticular manifestations (arthritis, arthralgias/myalgias); S, serositis (pleuritis, pericarditis); PN, proliferative nephritis; n-PN, non-proliferative nephritis; ND, not determined.

*WHO grade IV 10 yr prior to investigation, at inclusion no sign of renal exacerbation apart from stable proteinuria.
ELISA

ELISA was performed on frozen serum samples, which had been drawn and separated at the time of the ELISPOT investigations and then stored at −70°C until use. The anti-C1q ELISA was performed as described previously [8, 14]. According to the original description of the assay, the use of high-ionic-strength sample diluent buffer excludes antibodies reacting with immune complexes and only recognizes antibodies that specifically react with surface-bound C1q [14]. The concentration of a standard serum was arbitrarily defined as 1000 U/ml of IgG or IgA anti-C1q, respectively. As a control, sera from 40 healthy blood donors were investigated for the presence of IgG and IgA anti-C1q.

For IgG anti-dsDNA determination, a commercial kit (Dako, Glostrup, Denmark) was used according to the manufacturer’s instructions, having a limit of 25 IU/ml defined as the lower limit for a positive reaction.

Histopathology

Renal biopsies were graded according to the WHO classification [15].

Statistics

For statistical analyses, Fisher’s exact test, Mann–Whitney’s U-test and Spearman rank correlation were used. A P value of <0.05 was considered significant.

RESULTS

Clinical and histopathological characteristics of the patients with SLE and nephritis

Thirteen of the 21 patients had clinical signs indicating renal involvement and were all subjected to renal biopsy. Active glomerulonephritis was confirmed by histopathology in 11 patients. In two patients, the biopsies revealed renal sclerosis without signs of nephritis. One patient had nephritis WHO grade II, one grade III, five grade IV and four grade V. The remaining eight patients had active disease exacerbations with at least one non-renal organ involved. For more detailed information, see Table I.

The 21 patients were divided into three groups, one with PN (WHO grade III/IV), one with nephritis of non-proliferative type (n-PN; grade II + V) and a third with no laboratory signs of renal involvement or with renal sclerosis at biopsy [the non-nephritic (nN) group]. For age and nephritis characteristics in the patient groups, see Tables I and II.

Disease activity, estimated as SLAM scores, was evaluated from patient charts either prospectively or retrospectively. The median SLAM value for all patients was eight. No significant difference in SLAM index was observed between the patient groups (data not shown).

The patients with nephritis (PN and n-PN groups) received marginally higher doses of corticosteroid treatment and were more often treated with azathioprine at the time of investigation than the nN patients. Details of ongoing treatment are displayed in Table II.

ELISPOT

IgG anti-C1q SFC were observed in 15 of the 21 patients, levels ranging from 1 to 95 SFC/10^5 plated PBMC. In the PN group, the median value was 50
was an overlap (Table I). Very high titres (anti-C1q in PN patients and patients with no PN, there was an absolute relationship between the occurrence of PN and the other patients when the groups were compared (P = 0.027).

Serum levels of IgA anti-C1q that exceeded the reference range (367 U/ml) were observed in 2/21 investigated patients, of whom one had PN. There was no statistical difference between the PN group and the other patients when the groups were compared (P = 0.28; Mann-Whitney’s U). For details, see Table I.

Finally, concerning the serum levels of anti-dsDNA, 18/21 patients in the total group showed levels above the reference range. The patients with PN had higher levels than the other groups (P = 0.0018; Mann-Whitney’s U). There was, however, a substantial overlap between patients from different groups. (Table I).

**DISCUSSION**

Anti-C1q antibodies represent one of many types of autoantibodies reported in association with SLE. The occurrence of anti-C1q has been described in a substantial proportion of patients in mixed SLE populations and is associated with disease exacerbations, especially with nephritic manifestations [3–6]. In a recent study, increased serum levels of anti-C1q were also reported to precede and to herald proliferative nephritic disease flares, a finding which might indicate the antibodies to be of pathogenetic importance in this disease process [3].

Serological measurements have limitations, however, when used to measure autoantibodies. The binding of autoantibodies in vivo to the corresponding autoantigen may lessen the amount of free antibody available for detection in the circulation. In such cases, the ELISPOT method offers an advantage by measuring the present production of antibodies from cells in a cell culture system; hence, the total level of ongoing antibody production in the circulation can be estimated and compared in individual patients. However, the absence of anti-C1q-producing cells in the circulation does not exclude the presence of such antibody production elsewhere, such as in the spleen or bone marrow. The results of the ELISPOT assay performed on peripheral blood cells do consequently mirror both migration and antibody production of B cells.

One aim of the present study was to analyse to what extent production of anti-C1q in peripheral blood correlated with the occurrence of PN in SLE patients. A second question was to determine whether there was a discrepancy between the production of anti-C1q and the occurrence of unbound, and thus measurable, serum antibodies. If ongoing anti-C1q production, in comparison with the occurrence of serum antibodies, showed a stronger association with PN, this could provide some evidence for a pathogenetic role of these antibodies in the development of PN.

Concerning the occurrence of specific antibody production to C1q in peripheral blood of SLE patients, there was an absolute relationship between the occurrence of >20 IgG anti-C1q SFC/10^5 PBMC and the occurrence of biopsy-verified PN in the present study.

The patients with PN all had high numbers of IgG anti-C1q SFC (>20/10^5 PBMC), whereas the other patients had no or low numbers. The difference was strongly significant (PN vs n-PN + nN; P < 0.0001; Fisher’s exact test). For details of numbers of anti-C1q SFC, see Tables I and III.

IgA anti-C1q SFC were recorded in 10 of the 21 patients, levels ranging between 1 and 50 SFC/10^5 plated PBMC. In the PN group, 4/6 patients demonstrated ongoing anti-C1q production, in the n-PN group 2/5 and in the nN group 4/10. No statistically significant difference was observed, however, between the PN group and the other subgroups (P = 0.1; Mann-Whitney’s U-test). See Table I.

Total numbers of IgG, IgA and IgM SFC were recorded in 19/21 patients; in the other two patients, no results were available due to technical failure (high bluish background in patient no. 6, mistake in laboratory procedure in the analysis of total SFC in patient no. 8). No significant difference was observed between the PN group and the n-PN + nN groups concerning total numbers of IgG, IgA or IgM SFC. Compared to the healthy control group, however, numbers of IgG, IgA as well as IgM SFC were significantly increased in the SLE patients (P < 0.0001, P = 0.0051 and P = 0.0073, respectively, Mann-Whitney’s U). For details, see Tables I and III.

**ELISA**

Serum levels of IgG and IgA anti-C1q were determined by ELISA in all 21 individuals and in 40 healthy controls. In the control group, the mean titre of IgG anti-C1q was 33 U/ml and of the IgA 161 U/ml. The mean titre + 2 S.D. of the controls was regarded as the upper limit of normal; 67 U/ml for IgG and 367 U/ml for IgA anti-C1q.

Serum levels of IgG anti-C1q that exceeded the reference range (67 U/ml) were observed in 10/21 of the total number of investigated SLE patients. Concerning potential discriminatory capacity between levels of anti-C1q in PN patients and patients with no PN, there was an overlap (Table I). Very high titres (>450 U/ml) were only present in three patients with PN. On a group basis, there was a significant difference between the levels of IgG anti-C1q in the PN group as compared with the other patients (P = 0.039; Mann-Whitney’s U). Of the four non-nephritic patients with increased serum levels of IgG anti-C1q, one had 10 yr previously been treated for a nephritis WHO grade IV, however not giving evidence of ongoing renal involvement at the time of this study. Two other patients had no signs of nephritis during >6 months of follow-up, the fourth patient died.

A significant correlation between IgG anti-C1q ELISPOT and ELISA results was found for the total group of patients investigated (P = 0.0014; Spearman rank correlation). In subdividing the patient group, the correlation was found not to be significant for the PN and n-PN patients, whereas significance was obtained for the nN group (P = 0.027).

Concerning the occurrence of biopsy-verified PN in the present study, there was an absolute relationship between the occurrence of >20 IgG anti-C1q SFC/10^5 PBMC and the occurrence of biopsy-verified PN in the present study.
material. The increase of anti-C1q production in PN was determined to be largely confined to the IgG isotype, although single patients showed a few IgA anti-C1q SFC. The anti-C1q production also seemed to be selectively increased, and not only part of a general polyclonal B-cell activation. This conclusion could be drawn since no or few IgG anti-C1q SFC were recorded in patients without PN, despite the fact that these patients had similarly high numbers of total IgG SFC compared to the patients with PN.

There was a discrepancy, both at a group level and even more so in single patients, concerning the relationship between anti-C1q production as measured by ELISPOT and serum levels of anti-C1q (Table I). Even though there was a correlation between serum levels and numbers of anti-C1q SFC in the total study population, the correlation was not significant in any of the two subgroups of nephritic patients. Five out of six of the patients with PN, and 6/11 of patients with nephritis of any kind, had serum levels of IgG anti-C1q above the limit defined by normal donors. Conversely, 4/10 patients without signs of nephritis showed IgG anti-C1q levels above the reference range. On a general level, there was a better correlation between PN and ongoing anti-C1q production measured by ELISPOT than with anti-C1q serum levels measured by ELISA. Even more marked was the disparity in one single patient with PN (Table I, no. 1), in whom the serum level of IgG anti-C1q was within the reference range despite high anti-C1q production and florid nephritis. Whether this was due to binding of the antibodies to C1q and subsequent immune complex formation and/or elimination of the antibodies by other means is as yet unknown, but the possibility that such antibodies are particularly readily sequestered in the kidney obviously deserves consideration [3].

A tempting hypothesis for a pathophysiological function of anti-C1q would be that the antibodies might interfere with and possibly block the immune complex-clearing function. This could thereby cause an impaired function of the classical complement cascade similar to the impaired function observed in patients with hereditary complement deficiency syndromes. However, there are at present no empirical data that have directly been able to support such a hypothesis. In vitro, different monoclonal antibodies reactive with C1q have been reported either to activate complement [16] or block events included in complement activation [17]. This indicates that further analyses of the fine specificity of anti-C1q occurring in SLE will be necessary to prove definitely the idea of a pathogenetic role of functional impairment of C1q in SLE.

In summary, two findings are reported which may be of clinical importance. First, the ELISPOT data show that IgG anti-C1q production has a very high correlation with the occurrence of active proliferative SLE nephritis. These findings thus add to results from a number of previous investigations on the correlations between serum antibodies to C1q and PN [3, 5, 6], as to a possible pathogenetic importance of the IgG anti-C1q. Secondly, the highest serum levels of both IgG anti-C1q and anti-dsDNA were both observed in patients with PN, an observation which can be of clinical help in the evaluation of patients with suspicion of PN.

Finally, besides providing additional arguments for a pathogenetic role of anti-C1q in SLE nephritis, it is also feasible that measurement of antibody production by the ELISPOT method or by some other simplified procedure may also transpire to be a useful tool in the monitoring of patients with suspected lupus nephritis in clinical practice.

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