Predictive value of IgG autoantibodies against C1q for nephritis in systemic lupus erythematosus

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Abstract

Objectives—Antibodies against C1q (C1qAb) have been demonstrated in the serum of patients with several immune complex diseases. Patients, particularly those with lupus nephritis, were found to have increased serum titres of IgG C1qAb in a cross-sectional analysis. In the present prospective study correlations were sought between serum titres of IgG C1qAb and clinical as well as laboratory parameters of disease activity in patients with systemic lupus erythematosus (SLE).

Methods—Titres of IgG C1qAb in the serum of 68 SLE patients were measured serially during a three year period. At the same time clinical and laboratory parameters of disease activity were assessed.

Results—Increased titres of IgG C1qAb were found in the serum of 56% of SLE patients during the study. Significant correlations were found between increased titres of IgG C1qAb and renal involvement. Clinical signs of renal involvement were found to be associated with significant increases of serum titres of IgG C1qAb in the six months preceding this appearance. Fifty per cent of the increases in serum titres of IgG C1qAb were followed by the development of renal involvement. Elevated serum titres of IgG C1qAb were especially related to proliferative forms of glomerulonephritis. Furthermore, significant correlations were found between serum titres of IgG C1qAb and serum levels of immune complexes, levels of complement components, and titres of antibodies to DNA.

Conclusions—The results suggest that IgG C1qAb play a pathogenic role in the development of lupus nephritis and that serial measurement of serum titres of IgG C1qAb is useful in the management of SLE patients.

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Autoantibodies to C1q (C1qAb) have been reported in diseases in which immune complexes are considered to play a pathogenic role, such as systemic lupus erythematosus (SLE), rheumatoid vasculitis, and membranoproliferative glomerulonephritis.^{1–5} C1qAb of both immunoglobulin classes G and A have

been described.^{1–4} The results of a cross-sectional study suggested that serum titres of IgG C1qAb are associated with renal involvement in SLE patients.⁶ The aim of the present study was to further investigate associations between serum titres of IgG C1qAb in SLE patients and clinical as well as laboratory parameters of disease activity in a prospective study.

Materials and methods

PATIENTS

Sixty eight patients visiting the outpatient clinic of the department of rheumatology were studied prospectively between April 1988 and May 1991. All patients met the criteria for the classification of SLE.⁷ The mean period between the moment of diagnosis and the moment of study entrance was 6 years (range: 1 month to 24 years). There were 65 female and three male patients. The mean age of the patients at the start of the study was 38 years (range: 14–75 years). Clinical parameters of disease activity were recorded every six months by one observer (FCB). At the same time blood samples were obtained for the determination of laboratory parameters of disease activity. Serum samples for the measurement of titres of IgG C1qAb and of antibodies against double-stranded DNA (DNA-Ab) titres were stored in aliquots at –20°C until use. Four patients could not be evaluated for the complete study period.

CLINICAL PARAMETERS

Clinical parameters of disease activity were scored according to organ involvement. Erythema, photosensitivity, oral ulcers, discoid lesions, vasculitis, or alopecia were recorded for the assessment of skin involvement. Arthritis was recorded for the assessment of joint involvement, and pericarditis, pleuritis, or peritonitis for the assessment of serosa involvement. Proteinuria (+++ or >0.5 gr/24 h), haematuria (>10 red blood cells/high power field), or a decreased creatinine clearance (<100 ml/min) were recorded as manifestations of renal involvement. Seizures, cerebrovascular accidents, myeloneuropathy, or severe personality disorders were recorded for the assessment of central nervous system (CNS) involvement. A cumulative score for general disease activity (SLE-DAI) was used as well.⁸ During the study non-steroidal anti-

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inflammatory drugs, corticosteroids, hydroxy-chloroquine, and cytostatic drugs were taken by 29%, 76%, 40%, and 46% of the patients, respectively.

LABORATORY PARAMETERS

Levels of complement components C1q, C4, and C3 as well as total functional complement activity (CH50) were measured according to described methods.⁹ Levels of circulating immune complexes were measured using the fluid phase C1q binding assay (C1qBA) and expressed a $\mu\text{g/ml}$ of aggregated IgG.^{10, 11} Levels of DNA-Ab were measured using a *Crithidia lucilae* assay and expressed in titres of two-fold dilutions, starting at a dilution of 1/10.¹² A significant increase of serum titres of DNA-Ab was defined as at least a doubling of the titre (initial titre at least 1/10). Titres of DNA-Ab were also measured with the Farr assay in the serum of patients who developed renal involvement. In this assay bound DNA-Ab are precipitated by ammonium sulphate and the results were expressed in international units per millilitre serum (IU/ml) (Diagnostic Products Los Angeles, CA).^{13, 14} The upper limit of normal values in this assay was 15 IU/ml and a significant increase in DNA-Ab titres was defined as an increase of at least 25% and at least 15 IU/ml.

C1qAB MEASUREMENT

Titres of IgG C1qAb were measured by ELISA under 1M NaCl conditions. In this assay only IgG antibodies to the collagen-like region of C1q are detected. Neither immune complexes nor rheumatoid factors influence the assay results.⁴ Titres are expressed as U/ml related to a standard serum. The mean IgG C1qAb titre + 2 standard deviations of serum samples of 80 healthy blood donors was regarded as the upper limit of normal. This value was 90 U/ml. A significant increase of IgG C1qAb titres was defined as an increase of at least 25% and at least 90 U/ml. Total IgG levels were measured by ELISA in which wells were coated with mouse monoclonal anti-human IgG and subsequently bound polyclonal IgG in serum dilutions was detected by horse-radish peroxidase conjugated rabbit anti-human IgG.

RENAL BIOPSIES

In 20 patients a biopsy had already been obtained at a mean time of three years before the start of the study (range: two months to 14

years). In five patients a biopsy was obtained during the study. Patients had a renal biopsy when clinically indicated. The World Health Organisation classification of lupus nephritis was used to describe the histological lesions.¹⁵ Mixed forms of glomerulopathy were classified according to the proliferative lesion present.

STATISTICS

The Chi-square, Fisher's exact test (for numbers <5), and the Spearman non-parametric rank correlation coefficient were used. P-values below 0.05 were considered to be significant. Since the same number of observations was made for each patient at fixed intervals, each observation was considered as a separate observation when the Spearman correlation coefficient was used. A total of 422 observations were made. Clinical parameters were scored as qualitative variables. Laboratory parameters were considered as quantitative variables. C1qAb titres were considered both as qualitative (normal and increased titres) and as quantitative variables.

Results

CLINICAL PARAMETERS

The clinical parameters of disease activity observed in 68 SLE patients at the start of the study as well as new disease manifestations observed during three years of follow up are presented in table 1. Haematuria and proteinuria, as manifestations of renal involvement, are also shown. Skin and renal involvement were disease manifestations with the highest prevalence at the start of the study. New clinical manifestations of organ involvement during follow up predominantly concerned the skin. Joint, serosa, CNS, and renal involvement occurred less frequently. New involvement of skin, joints or CNS occurred twice in 4, 2, and 2 patients respectively.

Increased serum titres of IgG C1qAb were found in the serum of 29 (43%) patients at the start of the study and in the serum of 38 (56%) patients at any time during the study. Comparison of increased serum titres of IgG C1qAb and clinical parameters of disease activity of SLE at study entrance demonstrated a significant association between titres of IgG C1qAb and renal involvement (table 2). Similar associations were found between the presence of increased serum titres of IgG C1qAb and haematuria as well as proteinuria. No association was found between the presence of increased titres of IgG C1qAb and the involvement of other organs at the start of the study. Increased titres of IgG C1qAb measured at fixed intervals during three years of follow up were significantly correlated with the SLE-DAI score for general disease activity which were measured at the same time ($r = 0.18$; $p < 0.01$). Comparison of increased serum titres of IgG C1qAb and the presence of organ involvement during three years of follow up only revealed significant associations between the presence of increased IgG C1qAb

Table 1 Clinical parameters of disease activity according to organ involvement of 68 patients with SLE at study entrance and during three years of follow up

Organ involvement	Number of patients (%) with organ involvement at study entrance	Number of episodes with new organ involvement during the study	Number of patients (%) with episodes with new organ involvement during the study
Skin	25 (37)	30	26 (38)
Joints	5 (7)	10	8 (12)
Serosa	5 (7)	7	7 (10)
Central nerve system	11 (16)	13	11 (16)
Kidney	21 (31)	7	7 (10)
Proteinuria	18 (26)	6	6 (9)
Haematuria	16 (24)	6	6 (9)

Table 2 Comparison of the presence of increased serum titres of IgG C1qAb with the presence of organ involvement at study entrance in 68 patients with SLE

Increased C1qAb titre	+	-	+	-	p value*
Presence of organ involvement	+	-	-	+	
Skin	7	23	20	18	0.11
Joints	2	36	27	3	0.64
Serosa	4	38	25	1	0.10
Central nerve system	3	31	26	8	0.22
Kidney	15	33	14	6	<0.01
Proteinuria	14	35	15	4	<0.001
Haematuria	12	35	17	4	<0.01

*Chi-square or Fisher's exact test.

titres and the occurrence of renal involvement ($r = 0.40$; $p < 0.001$). No association was found between serum titres of IgG C1qAb and the use of particular drugs for the treatment of SLE (data not shown).

LABORATORY PARAMETERS

Comparison between serum titres of IgG C1qAb and laboratory parameters of SLE activity demonstrated positive correlations between IgG C1qAb titres and serum levels of creatinine, levels of circulating immune complexes measured by the fluid phase C1qBA, and serum titres of DNA-Ab (table 3). Negative correlations were found between serum titres of IgG C1qAb and levels of complement components of the classic pathway as well as total functional complement activity (CH50). No correlation was found between serum titres of IgG C1qAb and the ESR, haemoglobin content, leucocyte count, platelet count, and total IgG levels.

RELATION BETWEEN THE DEVELOPMENT OF ORGAN INVOLVEMENT AND INCREASES IN SERUM TITRES OF IgG C1qAb

To investigate a possible pathogenetic role of IgG C1qAb in the development of disease manifestations of SLE, the association between the appearance of clinical signs of organ involvement and significant increases in serum titres of IgG C1qAb was explored during the six months preceding such appearances (table 4). A significant increase in titres of IgG C1qAb was observed 10 times in 10 patients. Increases in serum titres of IgG C1qAb were significantly associated with the development of renal involvement. Each patient who

Table 3 Correlation* between serum titres of IgG C1qAb and laboratory parameters of disease activity in 68 patients with SLE during three years of follow up

Laboratory parameter	Correlation coefficient	p value
ESR	0.06	0.18
Haemoglobulin level	-0.08	0.10
Leucocyte count	-0.02	0.61
Platelet count	0.01	0.74
Serum creatinine	0.12	<0.05
C1q	-0.19	0.001
C4	-0.17	<0.01
C3	-0.15	<0.01
CH50	-0.15	<0.01
Fluid phase C1qBA	0.21	<0.01
DNA-Ab	0.14	<0.01

*Spearman rank correlation coefficient.

Table 4 Comparison of increases in serum titres of IgG C1qAb with the development of organ involvement in the six months following this increase in 68 patients with SLE during three years of follow up

Increased C1qAb titre	+	-	+	-	p value*
Development of organ involvement	+	-	-	+	
Skin	4	36	6	26	0.59
Joints	1	51	9	9	0.56
Serosa	3	54	7	4	0.06
Central nerve system	0	47	10	13	0.10
Kidney	5	56	5	2	<0.001
Proteinuria	4	56	6	2	<0.01
Haematuria	5	57	5	1	<0.001

*Fisher's exact test.

developed renal involvement associated with the presence of increased serum IgG C1qAb titres had a significant titre increase preceding the first appearance of clinical manifestation. Two patients who did not have increased titres of IgG C1qAb developed renal involvement. No correlation was found between increases of serum titres of IgG C1qAb and new SLE manifestations in other organs.

The specificity, sensitivity, and predictive value of significant increases of serum titres of IgG C1qAb for the development of kidney involvement are present in table 5. Significant increases in titres of IgG C1qAb were followed by the development of kidney involvement in 50% of the cases.

BIOPSIES

Renal biopsies were obtained in 20 SLE patients before the start of the study and in five patients during the study (table 6). In most biopsies a focal segmental (Class III) or diffuse proliferative (Class IV) glomerulonephritis was found. In the serum of 72% of patients who had glomerular lesions classified as Class III or IV before the study, increased serum titres of IgG C1qAb were found at the start of the study. In two other patients with glomerular lesions classified as Class I and II, titres of IgG C1qAb at the start of the study were found to be within the normal range. Five patients had a renal biopsy during the study. Three biopsies were classified as Class IV and one biopsy was classified as Class III. The serum of these four patients contained increased titres of IgG C1qAb before and at the time of the biopsy. The biopsy of the other patient, who did not have increased titres of IgG C1qAb throughout the study period, was classified as Class IIA.

RENAL INVOLVEMENT AND TITRES OF DNA-AB Previous studies have reported a relation between serum DNA-Ab and renal involvement in SLE.^{14 16-18} We therefore focussed on levels of DNA-Ab as measured by the *C luciliae* assay and the Farr assay of the patients with renal involvement. Comparison of increased serum levels of DNA-Ab as measured by the *C luciliae* assay with renal involvement measured at all observation points throughout the study demonstrated a significant correlation ($r = 0.24$; $p < 0.0001$). Three of the seven SLE patients who

Table 5 The sensitivity, specificity, and predictive value (95% confidence interval) of a significant increase in serum titres of IgG C1qAb for the development of renal involvement or its manifestations proteinuria and haematuria in 68 patients with SLE during three years of follow up

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Renal involvement	71% (29–96)	92% (82–97)	50% (19–81)	97% (88–99)
Proteinuria	67 (22–96)	90 (80–96)	40 (12–74)	57 (88–99)
Haematuria	83 (36–99)	92 (82–97)	50 (19–81)	98 (91–99)

Table 6 Renal biopsies in 25 patients with SLE classified according to the WHO and related to the presence of increased serum titres of IgG C1qAb during the study

WHO class	Biopsies before follow up	Increased C1qAb titre at study entrance	Biopsies during follow up	Increased C1qAb titre at the time of biopsy
I	1	0	0	0
II A	1	0	1	0
II B	0	0	0	0
III	4	2	1	1
IV	14	11	3	3
V	0	0	0	0

developed renal involvement during the study had increased serum DNA-Ab levels. However, significant increases in serum levels DNA-Ab before the development of renal involvement were observed less frequently compared with rises of serum IgG C1qAb titres. When employing the *C luciliae* assay, a significant titre increase was observed in only one of the patients and with the Farr assay such a rise was observed in the same and in two other patients. In all these three patients a significant rise of C1qAb titres was also observed.

Discussion

The existence of monomeric IgG that binds to C1q had already been noted a long time before the existence of IgG C1qAb was established. When studying the nature of immune complexes in SLE patients, several studies reported that not only immune complexes but also small ‘7S materials’ with characteristics similar to monomeric IgG bound to C1q.^{19–23} It was also noted that high titres of C1q binding ‘7S materials’ were frequently present in the serum of SLE patients and their presence was related to proliferative glomerular lesions.^{21–23}

The aim of our prospective study was to further investigate associations between serum titres of IgG C1qAb and disease manifestations of SLE. Significant associations were found between increased serum titres of IgG C1qAb and nephritis, with subsequent loss of kidney function. In addition, the development of nephritis was frequently preceded by a significant increase in serum titres of IgG C1qAb.

Many studies have investigated associations between disease activity of SLE and laboratory parameters. Levels of separate complement components,^{16–17–24–25} circulating immune complexes,^{17–24–26–27} and DNA-Ab^{14–16–18} were reported to be related to disease activity, but the results were not uniform. These laboratory parameters were most strongly related to lupus nephritis.^{16–18–26–27} The present study suggests that IgG C1qAb might be seen as a new

parameter for the development of lupus nephritis. As the appearance of renal manifestations associated with IgG C1qAb was always preceded by a significant titre increase and since the predictive value of increases of serum titres of IgG C1qAb for the development of kidney involvement was relatively high, the serial measurement of serum titres of IgG C1qAb might be useful in the management of SLE patients.

Measurement of DNA-Ab titres is used for the classification of SLE and in many centres as a laboratory parameter of disease activity. Several assays are available, each of which detects a part of the total spectrum of DNA-Ab.²⁸ The immunofluorescent assay using *C luciliae*, employed throughout this study, and the Farr assay are the most commonly used assays. Two recent prospective studies demonstrated that increments of DNA-Ab titres were associated with general disease activity and with the development of nephritis.^{14–18} Comparison between the *C luciliae* assay and the Farr assay demonstrated that the Farr assay was the most sensitive assay in this respect.¹⁴ The development of kidney involvement was preceded by increases in titres of DNA-Ab in nine of the 13 patients measured by the *C luciliae* assay and in 12 patients measured by the Farr assay. In the previous two studies serum samples for the measurement of DNA-Ab titres were obtained more frequently than in the present study, which, in addition to differences in the patient population, may be an explanation for the contrasting results.

C1qAb titres were correlated with several laboratory parameters known for their association with disease activity of SLE. The correlation between serum titres of IgG C1qAb and hypocomplementaemia as well as levels of circulating immune complexes may either indicate a contribution of IgG C1qAb to immune complex formation and subsequent complement consumption, or a direct influence on activation of the classic pathway of the complement system by IgG C1qAb. In a previous study we could not demonstrate that IgG C1qAb had a direct influence on activation of the classic pathway of the complement system in SLE patients.²⁹ However, it could be demonstrated that circulating immune complexes in the serum of patients with rheumatoid vasculitis contain C1qAb.⁴ The established correlation therefore between titres of IgG C1qAb and levels of circulating immune complexes and complement consumption indicates that IgG C1qAb in the serum of SLE patients contributes to immune complex formation.

Although C1qAb titres were measured in the majority of patients after renal biopsies was obtained, the association between IgG C1qAb and Class III and IV lupus nephritis may be important since the majority of the pathological diagnoses in lupus nephritis does not change with time.³⁰ The association found between IgG C1qAb and the proliferative form of glomerulonephritis in SLE patients studied both retrospectively and prospectively adds to

similar observations in lupus and other forms of nephritis.^{1 4 5 21 23 31} These observations suggest a pathogenic role for IgG C1qAb in the development of glomerulonephritis. Immune complexes are generally considered to play a pathogenetic role in lupus nephritis.³² Microscopic studies demonstrated the presence of immunoglobulins, C1q, and other complement components in glomeruli of SLE patients who develop nephritis.³³ Deposition of immune complexes in the kidney is believed to result from either glomerular trapping of circulating immune complexes or from in situ formation of immune complexes by fixation of antibodies to endogenous or exogenous (planted) antigens on the glomerular basement membrane. The degree to which each mechanism is responsible for the development of lupus nephritis is uncertain. Circulating immune complexes are found in high titres in the serum of nearly all SLE patients with proliferative glomerulonephritis.^{21 23} These immune complexes were shown to contain C1q.³⁴ A possible way of in situ immune complex formation is preferential binding of immune complexes to the glomerular basement membrane on the basis of charge-charge interactions.³⁵ Cationic immune complexes were shown to deposit principally at anionic sites of the glomerular basement membrane.^{36 37} Cationic antigens may also deposit at anionic sites of the glomerular basement membrane before forming an immune complex.^{38 39} C1q is a cationic protein and may induce local immune complex formation in the presence of C1qAb once it is bound to a solid-phase in the kidney. Recent studies have demonstrated that C1qAb are able to bind to C1q that is present on the glomerular basement membrane in vivo.^{40 41} IgG C1qAb may thus play a role in the development of nephritis in SLE patients, either by a contribution to the formation of circulating immune complexes that deposit in the kidney or by local formation of immune complexes on the glomerular basement membrane.

In conclusion the present prospective study demonstrates an association between the presence of increased serum titres of IgG C1qAb and the development of proliferative glomerulonephritis in patients with SLE. The association in time between increases in titre and the development of kidney involvement suggests that serial measurement of serum titres of IgG C1qAb may prove to be a valuable tool in the management of SLE patients.

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