

Anti-nucleosome antibody: Significance in lupus patients lacking anti-double-stranded DNA antibody

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Abstract

Objective

To investigate the clinical significance of anti-nucleosome antibodies in SLE patients lacking anti-double stranded DNA (dsDNA) antibodies.

Methods

IgG anti-nucleosome antibodies were detected by enzyme-linked immunosorbent assays (ELISA) in the sera of SLE patients. Anti-dsDNA antibodies were measured by Farr assays and ELISA, not only in the samples taken for anti-nucleosome testing, but also in sera obtained regularly during the follow-up.

Results

Ninety-eight (76.0%) out of 129 patients with SLE had anti-nucleosome antibodies. Twenty-five patients (19.4%) consistently showed little or no anti-dsDNA reactivity during the course of their disease, and among these anti-nucleosome antibodies were present in the sera of 15 (60.0%). Of the patients with anti-dsDNA-negative SLE, renal disorders were present in 8 patients (32.0%), all of whom had anti-nucleosome antibodies. Renal disorders were not found in patients (n = 10) who had neither anti-dsDNA nor anti-nucleosome antibodies. Other auto-antibodies such as anti-Ro, anti-Sm and anti-cardiolipin were not associated with renal disorders in this group. The levels of anti-nucleosome antibody strongly correlated with the SLEDAI scores, but inversely correlated with serum complement levels in anti-dsDNA negative SLE patients.

Conclusion

Our data suggest that the anti-nucleosome antibody may be a useful marker for diagnosis and activity assessment of anti-dsDNA negative SLE. Anti-nucleosome antibody may be an important factor for renal involvement in this subgroup of patients.

Key words

Anti-nucleosome antibody, anti-dsDNA antibody, SLE, renal disorder, disease activity.

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by a marked diversity of organ involvement and fluctuations in disease activity. Although the pathogenic process of SLE has not yet been clearly established, several autoantibodies have been implicated in its pathogenesis and in tissue damage (1, 2). In particular, anti-double stranded DNA (dsDNA) antibody is a classic autoantibody that characterizes SLE. Anti-dsDNA antibodies are disease-specific and closely associated with renal involvement and disease flares (3, 4). Furthermore, it is believed that anti-dsDNA antibodies may be involved directly in the pathogenesis of SLE through the formation of immune complexes leading to organ damage such as lupus nephritis (5,6). However, some SLE patients lack serum anti-dsDNA reactivity throughout the course of their disease. Nevertheless, they are not free from renal disorders and disease flares (7). Unfortunately, this is not clearly understood at present.

It is well known that DNA is not present in its naked form in the circulation of SLE patients, but is instead complexed with histones as a form of oligonucleosomes (8, 9). Moreover, the nucleosome acts as a major autoantigen for T cells, which are able to induce pathogenic autoantibodies in SLE (10-13). It was reported that circulating antibodies to nucleosomes might be associated with disease activity and renal involvement in SLE (14, 15). However, it is unclear whether anti-nucleosome antibodies are present in some SLE patients who persistently have no detectable anti-dsDNA antibodies throughout their disease course. We investigated whether anti-nucleosome antibody are to be found frequently in SLE patients lacking anti-dsDNA antibody persistently, and if so, whether it could be responsible for the renal disorders and disease activity in these patients.

Materials and methods

Patients and sera

This study included SLE patients who were treated at the Lupus Clinic of the Center for Rheumatic Diseases in

Kang-Nam St. Mary's Hospital from January 1997 to December 1999 and in whom the follow-up duration was more than 2 years (median follow-up 3.2 [range 2.2 - 5.7] years). Blood samples for anti-nucleosome antibodies were obtained from 129 SLE patients (7 males and 122 females, aged 12 - 66 years, median 29 years) and 50 healthy controls (2 males and 48 females, aged 18 - 52 years, median 27 years) in December 1999. Anti-dsDNA antibodies were measured in the same samples, and also in samples obtained regularly (median interval 16.5 weeks) throughout their disease course from the onset. The median number of sera samples tested for each individual was 13.5 (range 9 - 22). The SLE disease activity index (SLEDAI) (16), C3, C4 and urinalysis were also monitored regularly in all patients.

All patients fulfilled the American College of Rheumatism (ACR) criteria for SLE (17). Persistent proteinuria > 0.5 grams per day lasting for more than 6 months was defined as the existence of a renal disorder as previously described (17). Among a total of 129 patients with SLE, 57 (44.2%) had a renal disorder. Thirty-nine of them had undergone renal biopsy. On the basis of the World Health Organization (WHO) classification (18), 2 had class III lupus nephritis (LN) (focal segmental glomerulonephritis [GN]), 27 had class IV LN (diffuse proliferative GN) and 10 had class V LN (membranous GN). Active lupus was defined as a SLEDAI score > 5 as previously described (19). We defined 'anti-dsDNA positive SLE' as patients who showed anti-dsDNA antibody reactivities at least once during their disease courses, and 'anti-dsDNA negative SLE' as those who showed no or little anti-dsDNA reactivities persistently by both of two different assay systems.

Assays for antibody reactivities to nucleosome, dsDNA and other autoantigens

IgG anti-nucleosome antibody reactivities were assessed by enzyme-linked immunosorbent assays (ELISA, Medipan Diagnostica, Germany). This assay utilizes the intact nucleosome particles

purified from calf thymus as antigens to detect antibodies against all structural antigens of nucleosome. Patient sera were diluted 1:50 prior to the assay. Rabbit anti-human-IgG monoclonal antibody coupled with horseradish peroxidase was used as the detection antibody. The enzymatic reaction was carried out for a certain period of time to permit optimal color development. The optical density was read at 450 nm by an automated microplate reader (V_{max} , Molecular Devices, Palo Alto, CA). The antibody level was then calculated from a standard curve.

Anti-dsDNA antibody levels were measured by ^{125}I Farr assays using a commercial kit according to the manufacturer's specifications (Ortho-Clinical Diagnostics, UK). The sera of patients who did not show anti-dsDNA reactivity by Farr assays were re-evaluated by ELISA (Genesis Diagnostics Ltd, UK). The threshold for positivity was defined as 3 SD above the mean value of healthy controls (anti-nucleosome ELISA, 32.8 U/ml; anti-dsDNA Farr assay, 20.0 IU/ml; anti-dsDNA ELISA, 49.8 IU/ml). Other autoantibodies such as anti-Ro, anti-Sm and IgG anti-cardiolipins were determined by standardized commercial kits (anti-Ro by double immunodiffusion [DID; MBL Co., Nagoya Japan], anti-Sm by DID [MBL Co.], IgG anti-cardiolipin by ELISA [MBL Co.]).

Statistical analysis

Since the various data sets were not normally distributed, results are expressed as medians (minimum - maximum). Comparisons of numerical data were performed by Mann-Whitney U-test or Kruskal-Wallis test when appropriate, and matched pairs were analyzed by Wilcoxon's signed rank test. The chi-square test or Fisher's exact probability test was used to determine significant levels of observed frequencies. The correlations were determined by Spearman's rank correlation.

Results

Levels and frequency of antibodies to nucleosome and dsDNA

Ninety-eight (76.0%) out of 129 patients with SLE showed serum anti-

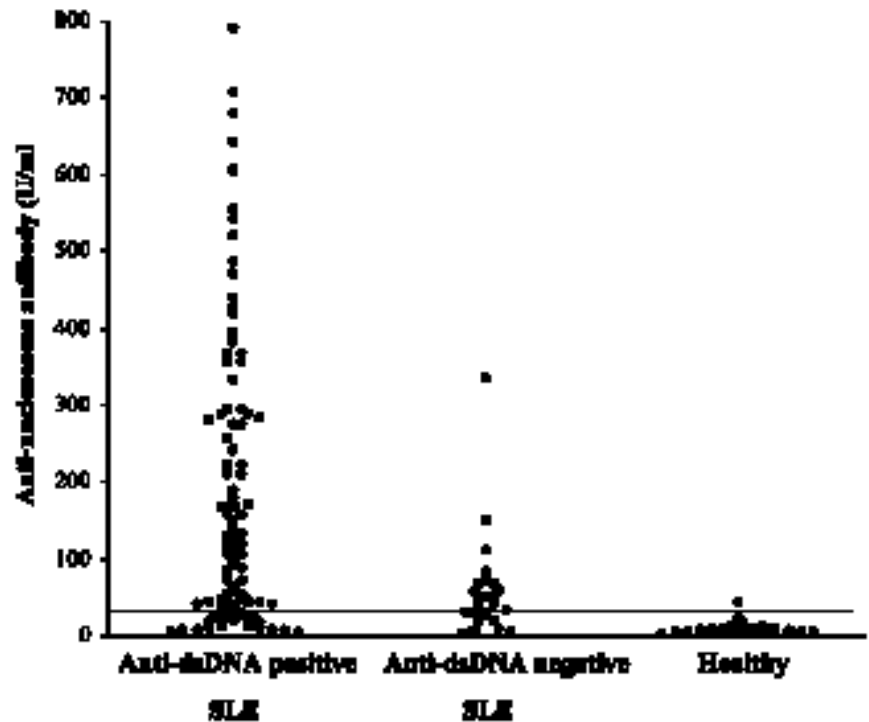


Fig. 1. Anti-nucleosome antibody reactivities. "Anti-dsDNA positive SLE" was defined as patients who showed anti-dsDNA antibody reactivities at least once during their disease courses, and "anti-dsDNA negative SLE" as those who did not. Anti-nucleosome antibodies were present in 79.8% of anti-dsDNA positive SLE, in 60.0% of anti-dsDNA negative SLE, and in 2% of healthy subjects, respectively. The broken line indicates the cut-off value.

body reactivity to nucleosome, whereas only 1 (2.0%) of 50 healthy controls had anti-nucleosome antibodies ($P < 0.001$). One healthy subject who showed minimal reactivity to nucleosome was a 52-year-old female. The circulating levels of anti-nucleosome antibodies in patients with SLE were significantly higher than those in healthy controls (median [range], 92.6 [3.6 - 791.0] versus 4.7 [2.9 - 44.0] U/ml, $P < 0.001$). Of the total 129 SLE patients, 25 (19.4%) lacked serum reactivity to dsDNA throughout the course of their disease by both of two different assay systems (anti-dsDNA negative SLE). Anti-nucleosome antibodies were present in 79.8% (83/104) of the anti-dsDNA positive SLE, and in 60.0% (15/25) of the anti-dsDNA negative SLE patients (Fig. 1).

Association of anti-nucleosome antibody with renal involvement

The levels of anti-nucleosome antibodies tended to be higher in patients with renal involvement ($n = 57$) compared to those with non-renal lupus ($n = 72$), but

this difference did not reach statistical significance (125.5 [6.0 - 791.0] versus 78.5 [3.6 - 681.0] U/ml, $P = 0.098$). Among the patients with anti-dsDNA positive SLE, the levels of anti-nucleosome antibodies were not significantly different between those with renal involvement and those without (133.0 [6.0 - 791.0] versus 96.0 [6.0 - 681.0] U/ml, $P = 0.486$). However, among the patients with anti-dsDNA negative SLE, patients with renal involvement showed significantly higher anti-nucleosome levels than those with non-renal lupus (62.9 [35.0 - 151.0] versus 31.0 [3.6 - 336.0] U/ml, $P = 0.023$) (Fig. 2A). Of this subgroup of anti-dsDNA negative SLE patients, renal disorders were found in 8 (32.0%), all of whom had anti-nucleosome antibodies. Conversely, lupus nephritis was not present in patients who had neither anti-dsDNA nor anti-nucleosome antibodies (Fig. 2B). Other autoantibodies such as anti-Ro, anti-Sm and anti-cardiolipin were not associated with renal disorder in this group. The frequency and levels of anti-nucleosome antibodies did not dif-

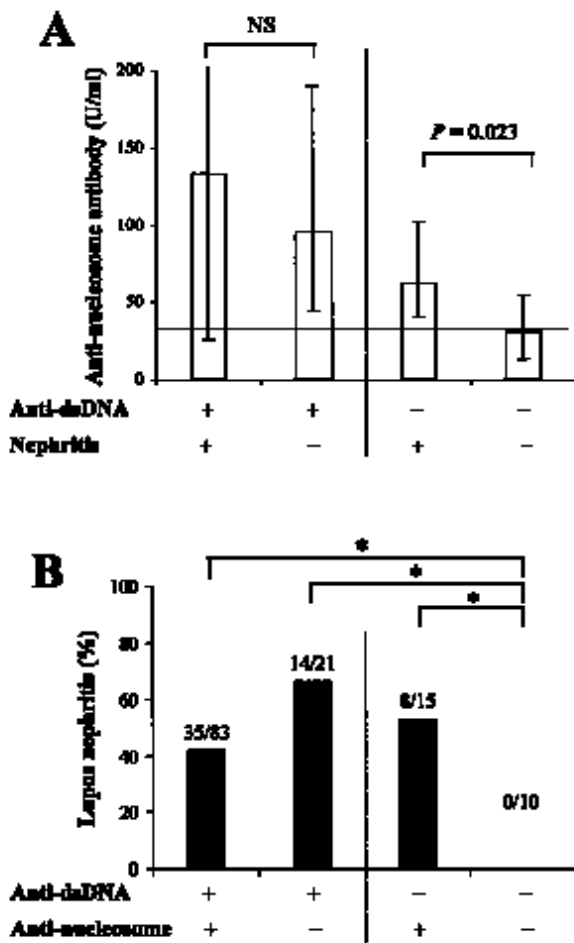


Fig. 2. Association of anti-nucleosome antibody with lupus nephritis.

(A) Anti-nucleosome antibody levels in relation to the presence of anti-dsDNA antibody and nephritis. Data are presented with the median and inter-quartile range. Horizontal broken line indicates the cut-off value. The definition of positive or negative anti-dsDNA is the same as in Figure 1.

(B) The prevalence of lupus nephritis according to the presence of anti-dsDNA and anti-nucleosome antibodies. NS: not significant, * $P < 0.01$.

fer with the WHO class of lupus nephritis. Other clinical manifestations of SLE were not correlated with anti-nucleosome antibodies (data not shown).

Correlation of anti-nucleosome antibody with disease activity

Patients with active SLE showed significantly higher levels of anti-nucleosome antibodies than those with inactive disease (151.5 [6.0 - 791.0] versus 52.5 [3.6 - 681.0] U/ml, $P = 0.002$). Levels of anti-nucleosome antibodies

strongly correlated with anti-dsDNA antibody levels ($r = 0.773$, $P < 0.001$) and with the SLEDAI scores ($r = 0.347$, $P < 0.001$), but inversely correlated with serum complement levels (C3, $r = -0.471$, $P < 0.001$; C4, $r = -0.360$, $P < 0.001$) in the whole group of SLE patients (Table I). The anti-dsDNA levels also correlated with the SLEDAI scores ($r = 0.390$, $P < 0.001$) and inversely with complement levels (C3, $r = -0.452$, $P < 0.001$; C4, $r = -0.357$, $P < 0.001$).

Table I. Correlation between anti-nucleosome Ab titers and other activity markers.

	In the entire SLE group (n = 129)		In anti-dsDNA negative SLE* (n = 26)	
	r	P [†]	r	P [†]
Anti-dsDNA	0.773	< 0.001	-	-
C3	- 0.471	< 0.001	- 0.523	0.007
C4	- 0.360	< 0.001	- 0.685	0.002
SLEDAI	0.347	< 0.001	0.449	0.024

* see Figure 1 for definition; † calculated by Spearman's rank correlation.

We next examined whether anti-nucleosome antibodies would reflect the disease activity in a subgroup of patients with anti-dsDNA negative SLE. The correlation of anti-nucleosome antibody levels with activity markers was also observed in patients with anti-dsDNA negative SLE (with SLEDAI scores, $r = 0.449$, $P = 0.024$; with C3, $r = -0.523$, $P = 0.007$; with C4, $r = -0.685$, $P = 0.002$) (Table I). Among these patients, those who had active SLE showed significantly higher anti-nucleosome antibody levels than those with inactive disease (58.9 [35.0 - 151.0] versus 31.0 [3.6 - 336.0] U/ml, $P = 0.031$).

To investigate whether anti-nucleosome levels would vary within the same individual during flare and remission, we examined paired samples of SLE patients, whose disease activity fluctuated markedly (change in SLEDAI > 5). The anti-nucleosome antibody levels were significantly increased during SLE flare in anti-dsDNA negative SLE patients ($P = 0.003$) (Fig. 3).

Discussion

The nucleosome is emerging as the most reactive substrate among the nuclear antigens in SLE, 70 - 80% of lupus patients being positive for anti-nucleosome antibodies (20-23). In this study, anti-nucleosome antibodies displayed a sensitivity of 76% in SLE, which is similar to that of previous reports. It has been strongly suggested that the nucleosome is the single primary target antigen for anti-dsDNA, anti-histone and anti-nucleosome antibodies in SLE (12, 22, 24, 25). Amoura *et al.* reported that 65% of their anti-dsDNA-negative sera showed anti-nucleosome antibody activity in a cross-sectional study of 120 SLE patients (12). However, anti-dsDNA levels can fluctuate and convert from positive to negative and vice versa during the disease course. It was unclear whether anti-nucleosome antibodies would be present in the sera of SLE patients who never show serum anti-dsDNA reactivity persistently. In the present study, the patients were regarded as having 'anti-dsDNA negative SLE' only when they showed no anti-dsDNA reactivity throughout the course of their

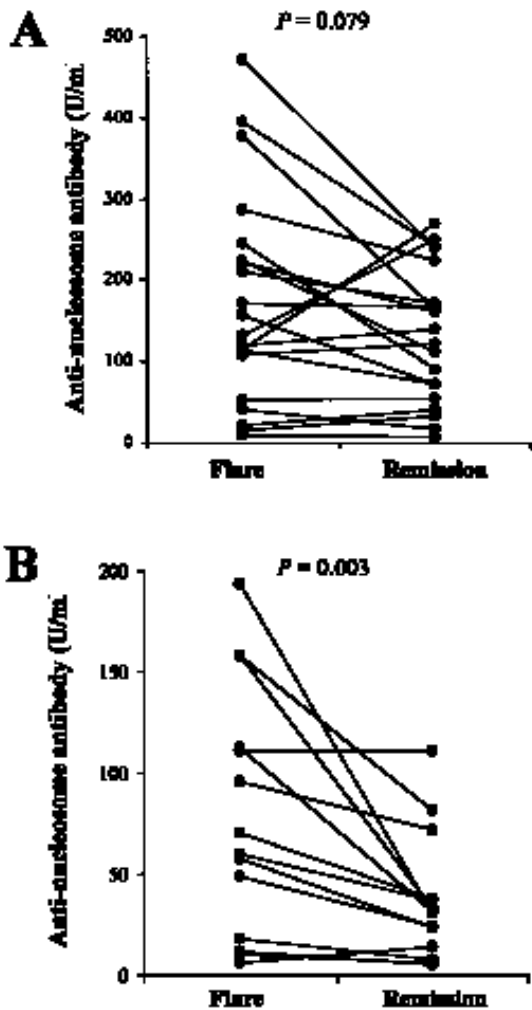


Fig. 3. Changes in the anti-nucleosome antibody levels during flare and remission within the same patients with anti-dsDNA positive SLE (A) and anti-dsDNA negative SLE (B). Paired samples were obtained from SLE patients whose disease activity fluctuated markedly (change in SLEDAI > 5).

disease for at least 2 years of follow-up since the disease onset. We found anti-nucleosome antibody reactivities in 60% of these persistently anti-dsDNA negative patients, supporting the view that the anti-nucleosome antibody may be a good marker for anti-dsDNA negative SLE.

Renal disorders are a cardinal manifestation of SLE, and affect the prognosis and mortality. Although the pathogenic mechanism leading to lupus nephritis is not clearly understood, anti-dsDNA antibodies are believed to be involved directly in the renal pathogenesis of SLE through the formation of immune complexes, eventually depositing on the glomerular basement membranes (5, 6). However, approximately one-third of SLE patients lack serum anti-dsDNA antibody reactivity throughout their disease courses, but are not free from renal disorders (7, 26). There is

insufficient evidence to support these explanations, although this may be due in part to the limited sensitivity of the detection methods for anti-dsDNA antibodies or to the possible pathogenicity of other autoantibody species such as anti-Ro or anti-Sm (27).

Recently accumulated evidence suggests that the nucleosome may be a major autoantigen for the pathogenic T cells and B cells of lupus (10, 13, 15). Moreover, there is strong evidence that anti-nucleosome antibodies play a role in the nephritogenic process; they are present in the kidney eluates of lupus mice with proteinuria (28), they bind to the glomerular basement membrane *in vivo* when complexed with nucleosomes (29), and they may correlate closely with proteinuria in SLE (14, 30). Thus, we investigated whether anti-nucleosome antibodies are relevant to the development of renal disorders in anti-

dsDNA negative SLE patients. We found herein that all patients who had a renal disorder in the absence of anti-dsDNA showed anti-nucleosome antibody reactivities, suggesting that anti-nucleosome antibodies may be responsible for the renal disorders in patients lacking anti-dsDNA antibodies. However, the association of anti-nucleosome antibody with renal disorder was not clear in anti-dsDNA positive patients. In addition, renal disorders were also frequently found in patients with anti-dsDNA but without anti-nucleosome, indicating that anti-dsDNA and anti-nucleosome antibodies may be involved in renal disorder independently (Fig. 2). To confirm whether lupus nephritis never develops in patients who have neither anti-dsDNA nor anti-nucleosome antibodies, further longitudinal studies are required.

Circulating anti-dsDNA antibody levels have been used clinically as a marker reflecting lupus activity, along with complement levels. In the present study, the levels of anti-dsDNA correlated with disease activity in the whole group of SLE patients, but not in cases where it was lower than the cutoff value throughout the course of their disease. It has been reported that anti-nucleosome antibodies reflect disease activity in patients with SLE (15, 21, 28). We have demonstrated here the usefulness of anti-nucleosome antibody levels as a valuable marker of disease activity, not only in SLE patients as a whole, but also in anti-dsDNA negative patients, by both cross-sectional and longitudinal analysis.

It remains a possible concern that anti-dsDNA and/or anti-histone antibodies may crossreact with the nucleosome antigens used for the anti-nucleosome assay, and thus be measured as positive anti-nucleosome. However, it was demonstrated in previous studies that most anti-nucleosome positive sera showed persistent antibody reactivity with little reduction in intensity even after depletion of dsDNA-specific and/or histone-specific antibodies using solid phase adsorption (14, 22). Moreover, we confirmed the absence of anti-dsDNA antibodies by more a sensitive assay - the ELISA - in the sera of patients who did

not show anti-dsDNA reactivity on Farr assays.

In conclusion, anti-nucleosome antibodies are detectable in more than half of patients with anti-dsDNA negative SLE, and may be responsible for renal involvement in these patients. Anti-nucleosome antibodies may also be a useful marker of disease activity in anti-dsDNA negative SLE patients. Our results emphasize the practical usefulness of anti-nucleosome antibodies, particularly in SLE patients whose reactivity to dsDNA is persistently absent.

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