

# Correlations between antinucleosome antibodies and anti-double-stranded DNA antibodies, C3, C4, and clinical activity in lupus patients

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## Abstract

### Objective

To examine the correlations between antinucleosome antibodies and anti-double-stranded (ds) DNA antibodies, complement (C) 3 and 4 levels, and clinical activities in SLE patients.

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### Methods

Antinucleosome antibodies and anti-dsDNA antibodies were detected by enzyme-linked immunosorbent assays (ELISA). The levels of C3 and C4 were measured by nephelometry. Clinical activities were determined by SLE Disease Activity Index (SLEDAI).

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### Results

Of 65 SLE patients, the prevalence of antinucleosome antibodies were higher than anti-ds DNA antibodies (52.3 vs 36.9%, respectively,  $p < 0.05$ ). Similar results were obtained in 45 active SLE patients, 64.4% for antinucleosome antibodies and 46.7% for anti-ds DNA antibodies. Of 34 patients lacking anti-ds DNA antibodies, 16 (47.1%) were shown antinucleosome antibodies. Activity of antinucleosome antibodies was significantly correlated with the SLEDAI scores and inversely correlated with the C3 levels but not with the C4 levels

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### Conclusion

Antinucleosome antibodies could be one of the earliest and most sensitive markers in diagnosis of SLE, particularly in anti-dsDNA antibodies-negative patients. More importantly, antinucleosome antibodies is correlated with clinical activities and C3 levels.

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### Key words

SLE, antinucleosome antibodies, anti-dsDNA antibodies, SLEDAI, C3, C4.

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## Introduction

Systemic lupus erythematosus (SLE) is a non-organ specific autoimmune disease characterized by widespread inflammation, affecting virtually every organs and/or systems in the body, and by the production of various autoantibodies, in particular, antinuclear autoantibodies (ANA). The ANA are autoantibodies directed against chromatin and its individual components including double-stranded DNA (dsDNA) and histones, and some ribonucleoproteins. Heretofore, anti-double-stranded DNA (anti-dsDNA) antibodies have been considered to be the serological hallmark that is disease specific and would participate in the development of various lesions in SLE (1-3). The primary event inducing the formation of anti-dsDNA, however, has always been obscured, since it has been very difficult to demonstrate the presence of free DNA in the sera of SLE patients (4). Furthermore, native DNA is usually considered non-immunogenic (5, 6); immunization with mammalian DNA could not induce pathogenic anti-dsDNA antibodies (7).

Currently, nucleosome, released by internucleosomal clearance of the chromatin during cell apoptosis, has been demonstrated in experimental as well as clinical levels to be the most reactive substrate among the nuclear antigens and plays a central role in both induction and pathogenicity of SLE including lupus nephritis (8-16). Antinucleosome antibodies are specifically induced by and react with only nucleosomes but not with its constituents DNA, and histones (2, 8). Furthermore, antinucleosome antibodies occur before the development of anti-dsDNA and anti-histone antibodies. Following studies have shown that antinucleosome antibodies poss high specificity for the disease (12, 13, 15) and could be positively detected in SLE patients lacking anti-dsDNA antibodies (17, 18). Of interest, the titers of antinucleosome antibodies excellently correlated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score (14, 19-21)

The correlations between antinucleosome antibodies and each serological

markers as well as clinical activities, however, have been separately studied in several previous studies (4, 5, 7, 14, 15, 17-23). As such, the present study was carried out to assess, in the same study, the correlation between antinucleosome antibodies and anti-dsDNA, C3 as well as C4 components of complement, and the SLEDAI score.

## Materials and methods

### *Patients and controls*

The study was approved by the Ethics Research Committee of the Faculty of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand. Each participating patient gave informed consent. Sixty-five patients from the outpatient and inpatient services of King Chulalongkorn Memorial Hospital, who fulfilled at least 4 of the American College of Rheumatology (ACR) revised criteria for SLE, were studied. To circumvent the drug effect on the levels of serologic markers (24), all the patients were newly diagnosed and did not receive corticosteroids and immunosuppressive drugs prior to the study. At each patient visit, clinical as well as biologic information was obtained and used to determine the SLEDAI score by the physicians. This was a blinded study, thus, the laboratory technician had no any information in disease activity of each individual patient before evaluating the correlation between serologic markers and disease activity. Active lupus was defined when the SLEDAI score was above 5 as previously described (25).

One hundred and eighty healthy blood donors (101 women, age range 17-53 years; mean 32 years; and 79 men, age range 19-58 years; mean 34 years) from the National Blood Bank Center; Thai Red Cross Society were collected and constituted as the control group.

Five milliliters (mL) of blood sample from each patient were collected, and stored at -80°C until used.

### *Preparation of nucleosomes*

Nucleosomes were basically prepared as previously described (12). Chicken erythrocytes were prepared from 1-2 mL of chicken blood by centrifuging at 800 g for 10 minutes at room tempera-

ture and the buffy coat was removed by suction. The erythrocyte pellet was washed 3 times with 10-fold excess, in a 15 mM Tris buffer containing 15 mM NaCl, 60 mM KCl, 2mM EDTA, 0.5 mM EGTA, 0.15 mg/ml spermine, 0.5 mM spermidine, 0.34 M saccharose, 15 mM 2-mercaptoethanol, and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), for 10 minutes, 2,000 rpm at 4°C. The cells were lysed in washing buffer containing 0.5% Triton X-100, using 10 mL of buffer/ml of red blood cells, incubated 5 minutes, 4°C, and centrifuged for 10 minutes, 2,500 g at 4°C. The pellets were washed 2 times in a digestion buffer containing 50 mM Tris, 25 mM KCl, 4 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, and 0.2 mM PMSF, using 10 ml of buffer/ml of red blood cells for 10 minutes, 2,000 rpm at 4°C. The pellets containing the nuclei were resuspended approximately in 1 mL of washing buffer/ml of starting volume of red blood cells and measured the concentration of DNA at OD 260 nm. Micrococcal nuclease (Sigma, USA) was added at the concentration of 40 IU/mg DNA and incubated at 37°C for 30 minutes and the reaction was terminated by addition of 0.2 M Na<sub>2</sub>EDTA to a final concentration of 2mM.

The nuclei were pelleted at 2,500 g for 10 minutes, 4°C, resuspended in 1 mL of an extraction buffer/ml of starting volume of red blood cells, and, then homogenized in a tight-fitting dounce homogenizer. The homogenate was dialyzed against extraction buffer overnight at 4°C and centrifuged for 10 minutes, 27,000 g at 4°C. The supernatant was subjected to a gel filtration column (Sephacryl S-300; Pharmacia, Freiburg, Germany) equilibrated in 50 mM Tris, 0.25 mM EDTA, 0.02% NaN<sub>3</sub>, and 0.2 mM PMSF, the volume should be in the range of 1-4% of the total bed volume of the column at a flow rate of 12 ml/h and collected the samples at 3 ml/fraction. Nucleosome fractions were collected by spectrophotometry after determination of the OD<sub>260</sub>. The fractions corresponding to pure mononucleosomes were concentrated on Amicon PM-30 filters (Amicon, Lexington, MA), and were stored at 4°C for no longer than 2 weeks.

Analysis by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated the presence of core histones composing H2A, H2B, H3 and H4. Furthermore, nucleosomal DNA was monitored by agarose gel electrophoresis showing the presence of nucleosomal DNA at 154 bp.

#### *Determination of antinucleosome and anti-dsDNA antibody*

Antinucleosome and anti-dsDNA antibody determination were assessed by indirect enzyme-linked immunosorbent assay (ELISA) as previously described (10). Briefly, antigen-coated plates (5 ug/ml for each antigen) were washed with phosphate buffered saline (PBS)-0.05% Tween 20, pH 7.4 and blocked with PBS-0.05% Tween 20 (PBST) and 10% fetal calf bovine serum (FBS) for 2 hours at room temperature. The sera to be tested in dilution of 1:100 in PBST-10% FBS were added to the plates and then allowed to react for 2 hours. The plates were washed with PBST and incubated with dilution of 1:4000 peroxidase-conjugated anti-human IgG for 2 hours at room temperature. Binding was measured by adding peroxidase substrate (o-phenylenediamine) and OD was read at 492 nm. Since standard serum expressing as international unit was not available in our laboratory, in the present study, the cut off values for discriminating positive and negative antinucleosome and anti-dsDNA antibodies were set at the mean OD of healthy control+3SD. Positive and negative control for both antibodies were included in each run of the assay.

The intra- and interassay coefficients of variation were 8.2% and 9.4%, respectively for antinucleosome antibodies and were 5.4% and 8.8%, respectively, for anti-dsDNA antibodies.

#### *Detection of antinuclear antibodies*

Antinuclear antibodies were detected by indirect immunofluorescence assay using HEp-2 cell (Diasorin, Stillwater, MN, USA) as the substrate. Antibody titer equal to or greater than 1:40 was considered positive.

#### *Complement assays*

The levels of C3 and C4 complements

were measured by nephelometry using the diagnostic kit (Behring AG, Marburg, Germany). The results were evaluated by comparison with a standard of known concentrations and the normal values for C3 and C4 were 76-171 and 10-40 mg%, respectively. CH50 was measured by standard hemolytic assay (normal 19-40 unit/mL).

#### *Statistical analysis*

Student's t-test was used for comparisons of the means. The chi-square test was used to determine the significant levels of correlations. The correlation analysis was analysed by linear regression. Statistical significance was considered when  $p < 0.05$ .

## **Results**

#### *Characterization of nucleosome preparations*

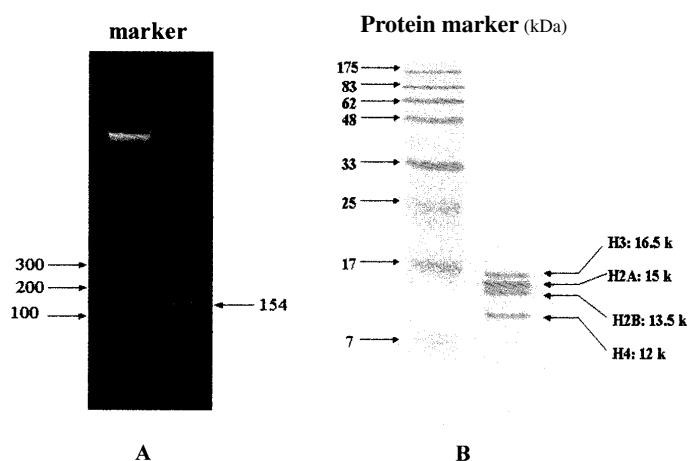
Preparation of nucleosomes was analyzed by gel electrophoresis. Nucleosomes were composed of DNA at 154 bp (Fig. 1A) and core histone bands at 16.5 kDa, 15 kDa, 13.5 kDa and 12 kDa for H3, H2A, H2B, and H4, respectively (Fig. 1B).

#### *Demographic data*

Of 65 participating patients, 63 were women (age range 16-62 years, mean  $\pm$  SE 33  $\pm$  4 years) and 2 were men (19 and 28 years). Forty-five patients had active SLE, the SLEDAI score above 5, while the remaining 20 subjects were inactive. Clinical presentations, according to the SLEDAI score, in patients with active SLE included alopecia (40%), new rash (22%), arthritis (20%), lupus headache (11%), mucosal ulcer (9%), pleurisy (9%), visual disturbance (7%), myositis (7%), fever (7%), organic brain syndrome (2%), and cerebrovascular accident (2%). By ACR criteria, lupus nephritis was identified in 36 patients.

#### *Detection and prevalence of antinucleosome and anti-dsDNA antibodies in SLE patients.*

The activities of antinucleosome and anti-dsDNA antibodies were determined in 65 SLE patients and 180 healthy controls. The values of mean  $\pm$  SD of antinucleosome antibody activity, re-



**Fig. 1.** Preparation of nucleosomes. (A) Nucleosomal DNA was present on agarose gel at 154 bp. (B) Nucleosomal histones on SDS-PAGE detecting 12 kDa (H4), 13.5 kDa (H2B), 15 kDa (H2A) and 16.5 kDa (H3).

vealed as optical density, were significantly higher in SLE patients than normal controls (SLE patients  $0.451 \pm 0.447$ , controls  $0.138 \pm 0.031$ ,  $p < 0.0001$ ). The similar results were also noted in the case of anti-dsDNA antibodies (SLE patients  $0.378 \pm 0.364$ , controls  $0.152 \pm 0.044$ ,  $p < 0.0001$ ).

Of 65 SLE patients, 34 (52.3%) were positive for antinucleosome antibodies and 24 (36.9%) were positive for anti-dsDNA antibodies ( $p < 0.05$ ). In contrast, none of the healthy controls were found to have these antibodies in their sera (Table I).

Next, the association between antinucleosome antibody activity and the disease activity were assessed. The levels of both antibodies, expressed as optical density, were depicted in Figure 2. Of 45 active SLE, 29 patient sera (64.4%) were positive for antinucleosome antibodies. In inactive SLE patients, 5 of

20 (25%) had antinucleosome antibody activities, indicating that active SLE patients had significantly higher positivity of these antibodies ( $p = 0.008$ ). Similar results for anti-dsDNA antibodies were also observed, that is, 21 (46.7%) of active SLE were found to have the antibodies while only 3 (15%) were positive in the inactive group ( $p < 0.01$ ) (Table I).

Table I also illustrates the results of antinuclear antibodies, which were present in almost all (63/65; 96.9%) of SLE patients (100% in the active and 90% in the inactive group, respectively).

#### Complement assays in SLE patients

As demonstrated in Table I, 19 of 65 SLE patients (29.2%) were shown to have low C3 levels (range 22.9–74 mg%). Of these SLE patients with low C3 levels, 18 were in the active SLE while the remaining one was inactive.

Low C4 levels (range 6.0–8.7 mg%), were noted in 9 patients (13.9%), all of whom were active.

CH50 levels were decreased in 27 SLE patients (41.5%), 24 of whom were active while the remaining three were in inactive state.

#### Association of antinucleosome antibodies with anti-dsDNA antibodies and complement C3, C4 levels

Of the 65 SLE patients, 18 (27.7%) exhibited both antinucleosome and anti-dsDNA antibodies, 16 (24.6%) were positive for only antinucleosome antibodies, 6 (9.2%) were positive for only anti-dsDNA, while the remainder 25 (38.5%) were all negative (Table II). It was interesting that the 16 patients who had only antinucleosome antibodies, 13 (81.3%) were in active state of SLE.

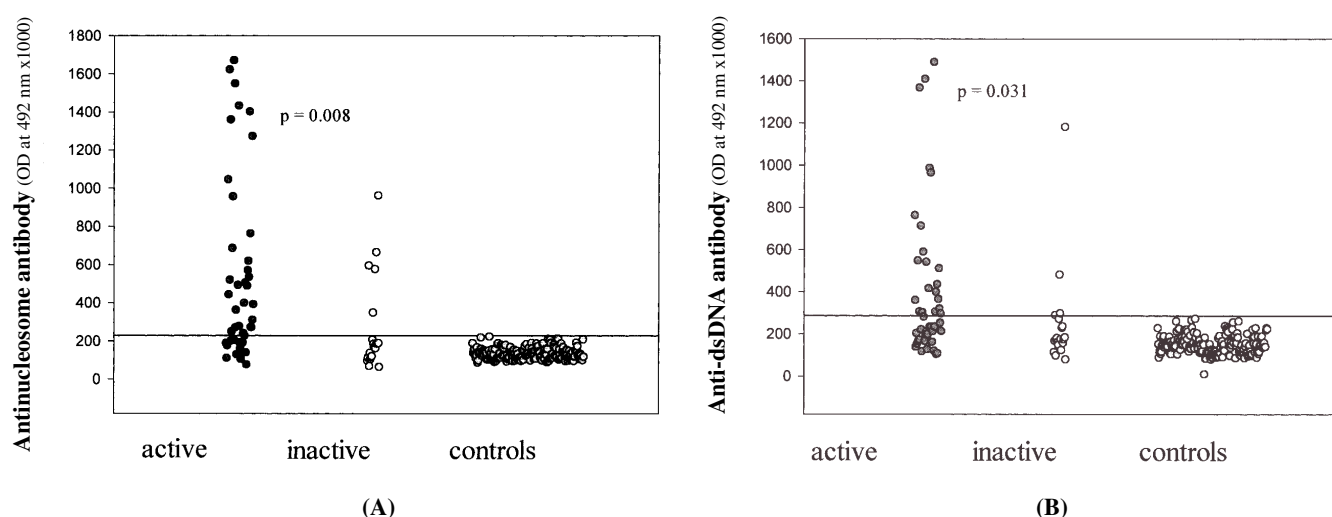
16/34 sera (47%) with antinucleosome positivity and 11/24 (45.8%) anti-dsDNA positive sera had low C3 levels. Of note, all sera which had low C4 (100%) were shown antinucleosome antibody activity. In contrast to anti-dsDNA positive sera, 6 of 8 (75%) sera with low C4 exhibited anti-dsDNA antibody activity (data not shown). In active SLE, the data revealed that 15 (51.7%) and 8 (27.6%) of 29 antinucleosome positive sera had low C3 and C4, respectively. In opposition to inactive SLE, only 1 (20%) of 5 antinucleosome-positive SLE sera had low C3. Of interest, none of the sera in this group revealed low complement C4 levels (data not shown).

To analyze the presence of anti-dsDNA in active SLE, the data revealed that 11 (52.4%) and 6 (28.6%) of 21 anti-ds-

**Table I.** Prevalence of antinuclear, anti-dsDNA, antinucleosome antibodies, and complement assays (C3, C4, and CH50) in SLE patients.

	No. tested	Presence of autoantibody <sup>#</sup> No. (%)			Decreased level of		
		ANA	Anti-dsDNA	antinucleosome	C3	C4	CH50
SLE							
Active	45	45 (100) <sup>NS</sup>	21 (46.7)*	29 (64.4) <sup>†</sup>	18 (40)	9 (20)*	24 (53.3)*
Inactive	20	18 (90)	3 (15)	5 (25)	1 (5)	0 (0)	3 (15)
Total	65	63 (96.9)	34 (52.3) <sup>†</sup>	19 (29.2)	9 (13.9)	27 (41.5) <sup>†</sup>	
Healthy controls	180	ND	0 (0)	0 (0)	ND	ND	ND

<sup>#</sup>ANA-antinuclear antibodies were detected by indirect immunofluorescence on HEP-2 cell, anti-dsDNA and antinucleosome antibodies were detected by ELISA, C3 and C4 levels was determined by nephelometry, <sup>NS</sup>: not significant, ND: not determined, \* $p < 0.01$  when compared with inactive patients, <sup>†</sup> $p < 0.05$  when compared with anti-dsDNA.



**Fig. 2.** The antinucleosome (A) and anti-dsDNA (B) antibody reactivities in SLE patients with active and inactive disease as measured by indirect enzyme-linked immunosorbent assay. The horizontal line shows the mean + 3SD optical density of healthy controls. P-values between active versus inactive SLE were obtained by the  $\chi^2$ -2 test.

DNA-positive sera had low C3 and C4 levels, respectively. In contrast to inactive SLE, none of the 3 anti-dsDNA-positive sera were found to have low complement C3 as well as C4 levels.

#### *Correlation between antinucleosome antibody activity and anti-dsDNA antibody activity*

In further examining the correlation between these 2 antibodies, the data demonstrated that the antinucleosome antibodies were significantly correlated with the anti-dsDNA antibodies in SLE patients ( $r = 0.82$ ,  $P < 0.0001$ ) (Fig. 3). The discrepancies of the results of these 2 antibodies were clearly observed in most of these patients, only very few were shown weakly positive (as indicated by OD) for antinucleosome antibodies with negative anti-dsDNA antibodies or vice versa.

#### *Correlation between antinucleosome, anti-dsDNA antibody activity and SLEDAI*

The simple regression analysis was used to identify the correlation between

antinucleosome and anti-dsDNA antibody activity, expressed as OD unit, and the SLEDAI score. Antinucleosome antibody activity in SLE was significantly correlated with SLEDAI ( $r = 0.33$ ,  $P = 0.007$ ) (Fig. 4A). Similar results were also noted between anti-dsDNA antibody activity and SLEDAI ( $r = 0.37$ ,  $P = 0.002$ ) (Figure 4B).

#### *Detection of antinucleosome, anti-dsDNA antibodies, C3, C4, and CH50 levels in SLE patients with and without lupus nephritis*

From the 65 SLE patients studied, 36 (55.38%) were found to have lupus nephritis. As shown in Table III, the presence of antinucleosome antibodies was significantly higher in lupus nephritis when compared to non-lupus nephritis patients (63.9% vs. 37.9%,  $p < 0.05$ ). No significant difference was noted in the presence of anti-dsDNA antibodies in both lupus nephritis and non-lupus nephritis patients. The levels of C3, C4, and CH50 were not significantly different between the both groups although the low levels of CH50 seemed to be a

more sensitive marker in lupus nephritis than in non-lupus nephritis group (47.2% vs. 34.5%, NS).

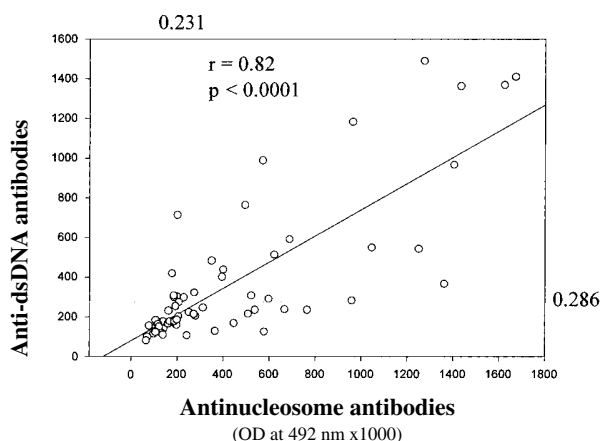
#### **Discussion**

Nucleosome is a large macromolecular complex comprised of a 154 base-pair DNA, wrapping around a core histone octamer, with a relative molecular mass of 250 kDa. Recent evidence obtained in murine models of SLE suggests that nucleosome is a preferential target for lupus autoantibodies and a putative autoantigen triggering the production of antibodies against its components, dsDNA and histones (22). This nucleosome is emerging as the most reactive substrate among the nuclear antigens in SLE, since 48-80% of SLE patients are found to have antibody response to nucleosomes (12-15).

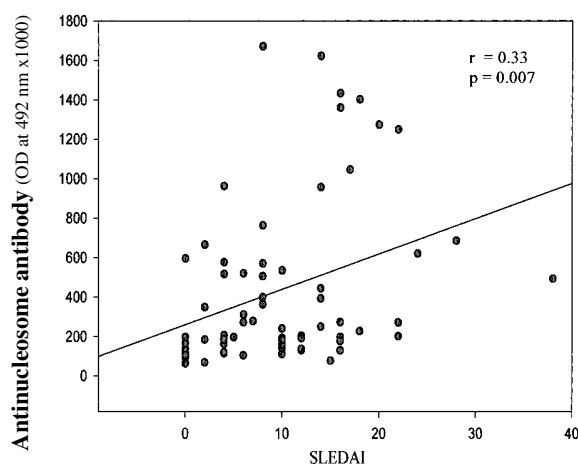
For a long time, "naked" dsDNA has been believed to be the major autoantigen in SLE. As a consequence, most of the studies on sensitivity and specificity have focused on anti-dsDNA antibodies. Nevertheless, dsDNA does not behave as such *in vivo* and also has poor immunogenicity in animal models (4-7). Indeed, DNA outside the cell is generally present in the form of nucleosomes generated by apoptosis (10, 16, 22). Thus, nucleosomes appear to be the particles that provide DNA *in vivo*, possibly making the DNA immunogenic when is not properly eliminated. Apoptosis defects are well known to be

**Table II.** Number (percentage) of antinucleosome and anti-dsDNA antibodies in 65 SLE patients.

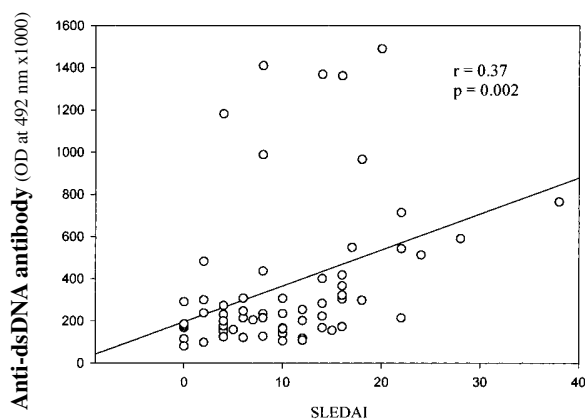
	Antinucleosome antibodies	
	Positive	Negative
Positive	18 (27.7)	6 (9.2)
Negative	16 (24.6)	25 (38.5)



**Fig. 3.** Correlation between antinucleosome and anti-dsDNA antibody activities. The OD cut-off (mean + 3SD) of antinucleosome and anti-dsDNA antibodies were indicated. Correlation analysis is performed by using linear regression.



**A** **Fig. 4.** Correlation between antinucleosome (A) and anti-dsDNA (B) antibody activity with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) in 65 SLE patients. Correlation analysis was performed using linear regression.



**B**

associated with certain animal models of lupus, and have also been discussed in connection with human SLE (10).

In the present study, the nucleosomes, prepared from the chicken erythrocyte nuclei, consisted of nucleosomal DNA banding at 154 base pairs and the core histone bands at molecular masses of 16.5k (H3), 15k (H2A), 13.5k (H2B), and 12k (H4) without the presence of

H1 linker protein (31k) or any other contaminant proteins. This indicates that this preparation yielded "intact" and highly purified mononucleosome core particles.

In this report, the correlation between antinucleosome antibodies, anti-dsDNA antibodies, C3, C4, and the SLEDAI score were simultaneously evaluated in 65 SLE patients and compared with

180 healthy controls. Antinucleosome antibodies were presented in 34 patients (52.3%). The results were similar to that of the previous works in which the figure of 48-80% had been reported (12-15). The frequency was lower for anti-dsDNA, 24 patients (36.9%) were found to be positive by the anti-dsDNA ELISA. Of the 180 healthy controls, none was revealed for antinucleosome as well as anti-dsDNA positivity.

The present data also suggest that the production of anti-dsDNA antibodies would be associated with that of antinucleosome antibodies (Fig. 3). One reason for this circumstance could be that most anti-dsDNA antibodies will also react with nucleosomes. Indeed, in the 24 anti-dsDNA positive SLE sera tested, anti-dsDNA activity was detected in concomitant with antinucleosome antibody activity in 75% (18/24) of patients. Interestingly, in SLE patients with anti-dsDNA negative sera, it had been reported that 60-65% of this group showed antinucleosome antibody activity (16,23). In the present study, 16 of 34 sera (47.1%) were shown to react with nucleosomes detected by ELISA without positivity for anti-dsDNA antibodies, supporting the view that the antinucleosome antibodies could be a reliable and accurate marker for anti-dsDNA negative SLE. However, 6 of 24 SLE patient sera (25%), which had anti-dsDNA antibody activity without exhibiting activity to nucleosomes, were present. According to the kinetic analysis of autoantibody production in lupus-prone mice, it had been demonstrated that the nucleosome-specific antibodies occurred early in the disease and preceded the formation of anti-dsDNA and anti-histone antibodies (11). In human SLE, of interest, changes in levels of anti-dsDNA, rather than absolute levels, have been shown to reflect new disease activity (2, 26, 27). It might be possible that the levels of these antibodies may fluctuate and convert from positive to negative and vice versa during the disease course. Further longitudinal studies are required.

Recent accumulating evidence suggests that antinucleosome antibodies are related to the SLE pathogenic pro-

**Table III.** Results of antinucleosome antibodies, anti-dsDNA antibodies, levels of C3, C4 and CH50 in SLE patients with and without lupus nephritis.

	Lupus nephritis (n = 36)		Non-lupus nephritis (n = 29)		P
Antinucleosome antibodies					
Positive	23	(63.9%)	11	(37.9%)	0.03
Negative	13	(36.1%)	18	(62.1%)	
Anti-dsDNA					
Positive	13	(36.1%)	11	(37.9%)	NS
Negative	23	(63.9%)	18	(62.1%)	
C3 level					
Low	11	(30.6%)	8	(27.6%)	NS
Normal	25	(69.4%)	21	(72.4%)	
C4 level					
Low	5	(13.9%)	4	(13.8%)	NS
Normal	31	(86.1%)	25	(86.2%)	
CH50					
Low	17	(47.2%)	10	(34.5%)	NS
Normal	19	(52.8%)	19	(65.5%)	

P values are lupus nephritis versus non-lupus nephritis patients. NS = not significant.

cess (10, 16, 21). In this regard, we found that, in 45 active SLE patients, 29 (64.4%) were positive for antinucleosome antibodies. Further analysis in inactive SLE patients revealed that only 5 of 20 (25%) patient sera showed antinucleosome antibody positivity. A previous study showed much higher occurrences of antinucleosome antibodies in active (100%) than inactive SLE patients (25%) (7). The higher frequency of active SLE than the present study might be explained by the much smaller sample size (n = 12 in active SLE group) used in the previous report. The evidence of participating in the pathogenic process of antinucleosome antibodies was emphasized by the higher frequency of this antibody found in lupus nephritis patients when compared to non-lupus nephritis group. The percentage of antinucleosome antibodies were also higher than other serologic markers (anti-dsDNA, C3, C4 and CH50 levels) in this study. The results suggested that detection of antinucleosome antibodies is superior to other markers in lupus nephritis. Further analysis for the isotype of antibody might be more meaningful, since it has been demonstrated that the antinucleosome IgG3 subclass is present at high levels in patients with active SLE but undetectable in inactive patients (15). The levels of IgG3 antinucleosome also

exerted a positive correlation with disease activity and were found to be closely associated with lupus nephritis. On the contrary, no significant correlation was observed between the disease activity and IgG3 anti-dsDNA (15).

In the present study, by using SLE Disease Activity Index (SLEDAI), both antinucleosome and anti-dsDNA antibody activities in SLE patients were comparable to have correlation to disease activity, ( $r = 0.33$ ,  $P = 0.007$  and  $r = 0.37$ ,  $P = 0.002$  respectively). In this regard, strong correlation was also noted between antinucleosome and anti-dsDNA antibody activities ( $r = 0.82$ ,  $P < 0.0001$ ). The present data are in agreement with many previous studies in which antinucleosome antibodies were found to correlate with the disease activity and anti-dsDNA antibodies (4, 5, 14, 17, 18, 22).

Recent studies have reported that only few connective tissue diseases in which antinucleosome antibodies are detected (12, 13, 15). Although, sera from other autoimmune diseases were not tested for antinucleosome antibodies in this study, the above findings in the present study are encouraging in respect of the practical use of the antinucleosome antibodies for the early diagnosis of SLE. Measurement of C3 and C4 levels together with their activity is widely used to assess disease activity in SLE (23).

That, in the present study, decreased C3 as well as C4 levels, are associated with active disease in SLE concurs with previous reports (23, 29, 30). In the present work, antinucleosome as well as anti-dsDNA antibodies were significantly correlated with C3 levels in SLE patients. No correlation was observed between antinucleosome antibody activity and C4 levels, although weak correlation has been previously reported (11). Interestingly, all 8 sera with low C4 levels in the current study had antinucleosome antibody activity.

The results in the present study emphasize the clinical application benefit of antinucleosome antibodies as one of the earliest markers for the diagnosis of SLE, especially in those that anti-dsDNA antibodies were undetectable. In the present study, except antinuclear antibodies, antinucleosome antibodies were the most sensitive marker in SLE patients when compared with other markers. Of more importance, their detection was significantly correlated with the disease activity. The use of antinucleosome antibody measurement as a marker of disease activity deserves further large-scale evaluation.

## References

1. HAHN BH: Antibodies to DNA. *N Engl J Med* 1998; 338: 1359-68.
2. ISENBERG D: Anti-dsDNA antibodies: still a useful criterion for patients with systemic lupus erythematosus? *Lupus* 2004; 13: 881-5.
3. NOSSENT HC, REKVIG OP: Is closer linkage between systemic lupus erythematosus and anti-double-stranded DNA antibodies a desirable and attainable goal? *Arthritis Res Ther* 2005; 7: 85-7.
4. RUMORE PM, STEINMAN CR: Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. *J Clin Invest* 1990; 86: 69-74.
5. PISETSKY DS: Systemic lupus erythematosus. *Curr Opin Immunol* 1991; 3: 917-23.
6. STOLLAR BD: The origin and pathogenic role of anti-DNA autoantibodies. *Curr Opin Immunol* 1989; 2: 607-12.
7. MADAIIO MP, HODDER S, SCHWARTZ RS, STOLLAR BD: Responsiveness of autoimmune and normal mice to nucleic acid antigens. *J Immunol* 1984; 132: 872-6.
8. TAX WJM, KRAMERS C, VAN BRUGGEN MCJ, BERDEN JHM: Apoptosis, nucleosomes, and SLE nephritis. *Kidney Int* 1995; 48: 666-73.
9. MONESTIER M: Autoantibodies to nucleosomes and histone-DNA complexes. *Methods* 1997; 11: 36-43.
10. AMOURA Z, CHABRE H, BACH JF, KOU-

- TOUZOV S: Antinucleosome antibodies and systemic lupus erythematosus. *Adv Nephrol Necker Hosp* 1997; 26: 303-16.
11. AMOURA Z, CHABRE H, KOUTOUZOV S *et al.*: Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/- mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum* 1994; 37: 1684-8.
12. BRUNS A, BLASS S, HAUSDORF G, BURMESTER GR, HIEPE F: Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. *Arthritis Rheum* 2000; 43: 2307-15.
13. WALLACE DJ, LIN HC, SHEN GQ, PETER JB: Antibodies to histone (H2A-H2B)-DNA complexes in the absence of antibodies to double-stranded DNA or to (H2A-H2B) complexes are more sensitive and specific for scleroderma-related disorders than for lupus. *Arthritis Rheum* 1994; 37: 1795-7.
14. CHABRE H, AMOURA Z, PIETTE JC, GODEAU P, BACH JF, KOUTOUZOV S: Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 1995; 38: 1485-91.
15. AMOURA Z, KOUTOUZOV S, CHABRE H *et al.*: Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases: antinucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum* 2000; 43: 76-84.
16. AMOURA Z, PIETTE JC, BACH JF, KOUTOUZOV S: The key role of nucleosomes in lupus. *Arthritis Rheum* 1999; 42: 833-43.
17. MIN DJ, KIM SJ, PARK SH *et al.*: Anti-nucleosome antibody: significance in lupus patients lacking anti-double-stranded DNA antibody. *Clin Exp Rheumatol* 2002; 20: 13-8.
18. AMOURA Z, CACOUB P, CHABRE H *et al.*: Prevalence of anti-nucleosome autoantibodies in connective tissue diseases (abstract). *Arthritis Rheum* 1997; 40 (Suppl. 9): S85.
19. AMOURA Z, PIETTE JC, CHABRE H *et al.*: Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: correlation with serum antinucleosome antibody titers and absence of clear association with disease activity. *Arthritis Rheum* 1997; 40: 2217-25.
20. BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
21. BURLINGAME RW, BOEY ML, STARKEBAUM G, RUBIN RL: The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J Clin Invest* 1994; 94: 184-92.
22. BURLINGAME RW, RUBIN RL, BALDERAS RS, THEOFILOPOULOS AN: Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. *J Clin Invest* 1993; 91: 1687-96.
23. SWAAK AJ, GROENWOLD J, BRONSVELD W: Predictive value of complement profiles and anti-dsDNA in systemic lupus erythematosus. *Ann Rheum Dis* 1986; 45: 359-66.
24. LEE CH, SUH CH, LEE J, KIM YT, LEE SK: The effects of anti-idiotypic antibody on antibody production and apoptosis of anti-dsDNA antibody producing cells. *Clin Exp Rheumatol* 2003; 21: 291-300.
25. TOUBI ERI, ROZENBAUM M, KESSEL A, GOLAN TD: The benefit of combining hydroxychloroquine with quinacrine in the treatment of SLE patients. *Lupus* 2000; 9: 92-5.
26. BOOTSMA H, SPRONK P, DERKSEN R *et al.*: Prevention of relapse in systemic lupus erythematosus. *Lancet*; 1995: 1595-9.
27. BORG EJT, HORST G, HUMMEL EJ, LIMBURG PC, KALLENBERG CGM: Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus: A long-term, prospective study. *Arthritis Rheum* 1990; 33: 634-43.
28. SINICO RA, BOLLINI B, SABADINI E, DI TOMA L, RADICE A: The use of laboratory tests in diagnosis and monitoring of systemic lupus erythematosus. *J Nephrol* 2002; 15 (Suppl. 6): 20-7.
29. SCHUR P, SANDSON J: Immunologic factors and clinical activity in systemic lupus erythematosus. *N Engl J Med* 1968; 278: 533-8.