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Anti-Histones Antibodies in Systemic Lupus Erythematosus: Prevalence and Frequency in Neuropsychiatric Lupus

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To investigate the specificity, sensitivity, and concomitant presence of antibodies against histones (H), histone H1 (H1), and histone H3 (H3) in patients with systemic lupus erythematosus (SLE) and analyze their association with SLE. Serum IgG anti-histones antibodies were detected by enzyme-linked immunosorbent assay in 144 SLE patients consisting of 24 neuropsychiatric lupus (NPSLE), 65 lupus nephritis (LN), and 55 SLE, 100 other rheumatic diseases patients, as well as 40 healthy controls. Clinical and biological parameters of the patients were also evaluated. Anti-H, anti-H1, and anti-H3 antibodies yielded a sensitivity of approximately 33% and a specificity of more than 93% for SLE, which was comparable to that found for anti-double-stranded DNA (anti-dsDNa) antibodies. More significantly, anti-histone antibody is found in approximately 50% of patients with NPSLE compared with LN. Moreover, the titers of anti-histones antibodies of NPSLE patients were significantly higher than that of patients with SLE and LN. The sequential analysis revealed a close correlation of anti-H and anti-H1 antibodies with SLE disease activity. There was an approximate 30% positive rate of anti-histones antibodies in 144 SLE patients lacking anti-nucleosome, anti-mDNA, anti-Sm, and anti-dsDNA antibodies. Antibodies to histones H1 and H3 are markers with high specificity of 93.6–96.4% for SLE. The anti-histone antibody markers are prevalent in approximately 50% of NPSLE. Furthermore, there was a strong correlation with SLE disease activity index and levels of antibodies to histones and H1. J. Clin. Lab. Anal. 22:271–277, 2008. © 2008 Wiley-Liss, Inc.

Key words: autoantibody; histones; systemic lupus erythematosus; neuropsychiatric lupus

INTRODUCTION

Systemic lupus erythematosus, termed as SLE, is a systemic autoimmune disease characterized by chronic inflammatory tissue damage mediated by a central part of the autoimmune response, which is directed against chromatin components (1–3). SLE can almost affect any part of the body, often harming the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. This immune response against chromatin components is a typical and to a certain extent a specific feature of SLE suggestive of a pathogenic role that is yet to be fully elucidated. Double-stranded (ds) DNA (4), histones (5,6), nucleosomes (7,8), as well as more complex antigenic structures resulting from the assembly of histones and nonhistone proteins with DNA (9) are recognized by both cellular and humoral constituents of the immune system in SLE patients.

Accumulating evidences indicate that immune complexes of specific antibodies, histones, and/or nucleosomes participate in the disease process (10–13), and the existence of antibodies against dsDNA is a highly specific feature in SLE patients and determination of such antibodies has been established as an important tool for diagnosing and monitoring of this disorder.

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Actually, antibodies to histones (AHA) are commonly seen in SLE patients. The known structures of the histones and the use of the solid-phase assays have allowed their nature to be defined in greater detail. Currently, however, determination of antibodies against histones may be underestimated. In addition, discrepant studies have been reported on the association of AHA with disease activity or severity with the predominant organ system affected or with specific clinical features (14–17).

This study is designed to perform a comparative analysis of antibodies to histones, H1, and H3 in SLE patients and also address the question whether these antibodies are useful for monitoring SLE patients. Furthermore, prevalence, specificity, and relationship to clinical and laboratory features of these antibodies in different subgroups of SLE, i.e., neuropsychiatric lupus (NPSLE), lupus nephritis (LN), and SLE, are compared. The data reveal that histones and their subclasses H1 and H3 are highly specific autoimmune targets in SLE, suggesting that anti-histone and anti-H1 test may represent a potent tool for assessment of SLE disease activity.

PATIENTS AND METHODS

Patients and Serum Samples

Serum samples were collected from 144 SLE patients (133 women and 11 men with a mean age of 34 years, ranging from 11 to 69 years) who had been hospitalized at our institution between February 1, 2007, and July 30, 2007. The mean disease duration was 5.8 ± 6.3 years. The patients fulfilled the 1997 revised SLE criteria of the American College of Rheumatology (18). Individual disease activity was quantified using the SLE disease activity index (SLEDAI) score. Active lupus was defined as a SLEDAI score >8 as previously described (19). Persistent proteinuria >0.5 g per day at two times was defined as the existence of a renal disorder (18,20). All NPSLE patients were evaluated according to a standardized protocol by the participating rheumatologists and neurologists at the time of hospitalization.

Control group was composed of 100 patients including 25 systemic sclerosis (SSc, mean age 56 ± 8.7 years, disease duration 7.8 ± 6.9 years), 28 rheumatoid arthritis (RA, mean age 51 ± 10.2 years, disease duration 4.2 ± 5.8 years), 10 Sjogren's syndrome (SS, mean age 54 ± 7.5 years, disease duration 6.5 ± 4.1 years), 14 polymyositis or dermatomyositis (PM/DM, mean age 51 ± 9.4 years, disease duration 6.7 ± 4.5 years), 12 ankylosing spondylitis (AS, mean age 20.5 ± 6.8 years, disease duration 5.5 ± 4.3 years), and 11 osteoarthritis (OA, mean age 53 ± 9.3 years, disease duration 7.8 ± 6.9 years) patients. Healthy control (HC) serum samples were obtained from 40 blood donors (mean age 48.5 ± 8.4 years, female (n)/male (n) = 28/12). All sera were kept at -20°C.

Clinical features of SLE patients such as skin rash, fever, ulceration, alopecia, pleuritis, arthralgia, photosensitivity, and systemic lesions were recorded. Complete blood cell count, routine examinations for urine, erythrocyte sedimentation rate, C reactive protein, IgG, IgA, IgM, and serum levels of C3 and C4 were detected.

Antibodies

ELISA for antibodies to histones, H1, and H3

Histones, H1, and H3 from calf thymus chromatin were purchased from Sigma (Shanghai, China) and verified by sodium dodecylsulfate polyacrylamide gel electrophoresis. Enzyme-linked immunosorbent assay (ELISA) testing of anti-histones, anti-H1, and anti-H3 antibodies was performed according to a previously described protocol with minor modifications (21). Briefly, the proteins were dissolved in 0.05 M carbonate buffer (pH 9.6) at a concentration of 5 μg/ml and coated onto polystyrene microtiter plates (Costar, Cambridge, UK) at a volume of 100 μl per well. After incubating for 6 hr at 37°C, the plates were washed four times with 0.1% Tween20/phosphate-buffered saline (PBS) and blocked with 5% dried skim milk/PBS overnight at 4°C. One hundred microliter serum samples diluted 1:200 were added to each well. After incubation for 2 hr at 37°C and washing, anti-human IgG conjugated to peroxidase was added to the wells. Then the bound antibodies were detected with the *O*-phenylenediamine dihydrochloride color-development reagent. Plates were read at absorbance of wavelength 492 nm (OD 492 nm).

Each serum sample was assayed in duplicate and the results were averaged. A pool of highly positive serum samples was included on each plate as a reference to correct interassay variations. To evaluate the ELISA data, the OD measurements were transformed into arbitrary unit (AU) and calculated in relation to the respective standard control of the positive serum: $AU = [\text{average } OD_{\text{histones}} \_ \text{average } OD_{BSA}]_{\text{test serum}} / [\text{average } OD_{\text{histones}} \_ \text{average } OD_{BSA}]_{\text{standard serum}} \times 100$.

Other specific antibodies

Anti-Sm antibodies were detected by immunoblotting. Autoantibodies to dsDNA and anti-nuclear antibodies (ANA) were detected by immunofluorescence method. (The antibodies were from Medizinische labordiagnostika GmbH, Lubeck, Germany.) A dilution of 1:40 was considered as a positive test. Anti-deoxyribonucleoprotein (DNP) antibodies were detected by Latex agglutination (Omega biocompany, Edinburgh, Scotland). Antibodies to cell membrane DNA (cmDNA) were

identified by an indirect immunofluorescence assay using a Rajii cell line (Beijing, China) fixed but not permeabilized as described previously (22).

Statistical Analysis

Data analyses were performed using SPSS 13.0 software for Windows (SPSS, Inc., Beijing, China). For normally distributed data, the results were expressed as mean $\pm$ SD and the differences in means between groups were analyzed with the *t*-test. For abnormally distributed data, expressed as median (range), the differences were tested with the Mann–Whitney *U*-test. The correlations were sought by computing Spearman rank correlation coefficients. The χ^2 test was used to compare percentages. Comparisons of categorical data between groups were analyzed with the McNemar χ^2 test, and the correlation between two variables was performed using Kendall's Turb correlation coefficients. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Autoantibodies to Histones in SLE

Antibodies to chromatin components in sera of patients with various rheumatic diseases including SLE, RA, SS, SSc, PM/DM, AS, OA, as well as in HCs were measured by ELISA (Table 1). Among the antibodies to histones, H1, and H3, anti-H3 antibody had a highest sensitivity (53/144, 36.8%), anti-histones antibody (48/144, 33.7%) was lower than anti-H3, and the antibody to H1 had the lowest sensitivity (37/144, 25.7%). In other patient groups, the three antibodies were only found in four to eight of 25 patients with SSc, none to two of 28 patients with RA, and one of the 14 patients with PM/DM; none of the patients with other rheumatic diseases (i.e., SS, OA, AS) nor any of the HCs gave a positive result (Table 1). The overall specificity of anti-H3 testing for SLE was 96.4%. Anti-H and anti-H1 shared similar specificity (93.6%). It is noteworthy that, in all positive non-SLE patients, levels of antibodies to H, H1, and H3 were relatively low.

ANA antibodies had a rather high sensitivity (65.9%), but, as expected, a lower specificity for SLE (86.4%) owing to the high proportion of positive samples within other rheumatic disease groups, even in one of the HCs. The sensitivity of antibodies to nucleosomes (AunA) was 52.8%, whereas their specificity was only 89.3% as they were also detected in most other disorders, with 21.4% (RA, 6/28) to 28% (SSc, 7/25) of the samples showing a positive result. In the cohort of patients, the sensitivity of anti-dsDNA antibodies (51/144, 35.4%) was similar to that of anti-H3, and that was significantly lower than their specificity (97.1%). The specificity of anti-Sm (99%) and anti-DNP (100%) was higher than other antibodies, but their sensitivities were very lower, of only 15.9 and 11.1%, respectively (Table 1).

Autoantibodies to Histones in Subgroups of SLE

In this study, 144 patients with SLE consisted of 24 patients with NPSLE, 65 patients with LN, and 55 systemic erythematosus. The positive rate of antibodies to chromatin components and other antibodies in subgroups of SLE is given in Table 2. Antibodies to histones, H1, and H3 had the most frequency in NPSLE group (58.3, 41.7, 50%) compared with LN group (30.9%, $P<0.05$; 25.5%, $P<0.05$; 38.2%, $P<0.05$) and SLE group (26.2%; 20%, $P<0.01$; 30.8%). Moreover, the AU of the three antibodies in patients with NPSLE was significantly higher than LN and SLE groups (Fig. 1).

A similar tendency was also observed in anti-dsDNA, anti-Sm, and anti-DNP antibodies (Table 2). On the

TABLE 1. Antibodies to Chromatin Components and Other Antibodies in Various Rheumatic Diseases

Disease	Total (*n*)	H	H1	H3	ANA	AnuA	dsDNA	Sm	cmDNA	DNP
SLE	144	48	37	53	95	76	51	23	54	16
RA	28	2	0	0	6	6	0	0	2	0
SSc	25	6	8	4	6	7	2	1	2	0
SS	10	0	0	0	2	1	1	0	2	0
AS	12	0	0	0	0	0	0	0	0	0
PM/DM	14	1	1	1	4	1	1	0	1	0
OA	11	0	0	0	0	0	0	0	0	0
HC	40	0	0	0	1	0	0	0	0	0
Sensitivity for SLE (%)		33.3	25.7	36.8	65.9	52.8	35.4	15.9	37.5	11.1
Specificity for SLE (%)		93.6	93.6	96.4	86.4	89.3	97.1	99.2	95	100

Antibodies to histones (H), histone H1 (H1), histone H3 (H3), and nucleosomes (AnuA) were measured by ELISA; anti-nuclear antibodies (ANA), anti-cell membrane DNA (cmDNA), and double-stranded DNA (dsDNA) were measured by immunofluorescence method; anti-Sm antibodies were detected by immunoblotting; anti-DNP antibodies were detected by Latex agglutination. The patients include systemic lupus erythematosus (SLE), systemic sclerosis (SSc), rheumatoid arthritis (RA), Sjogren's syndrome (SS), polymyositis or dermatomyositis (PM/DM), ankylosing spondylitis (AS), osteoarthritis (OA), and healthy control (HC). ELISA, enzyme-linked immunosorbent assay; *n*, number of patients.

TABLE 2. Antibodies to Chromatin Components and Other Antibodies in Subgroups of SLE

Subgroups	Anti-H	Anti-H1	Anti-H3	ANA	AnuA	Anti-dsDNA	Anti-Sm	Anti-cmDNA	DNP
SLE	17	13	20	40	29	24	8	21	7
$n = 55$	26.2%	20%**	30.8%	72.7%	52.7%	43.6%	14.5%**	38.2%	12.7%**
NPSLE	14	10	12	17	13	9	6	9	4
$n = 24$	58.3%	41.7%	50%	70.8%	54.1%	37.5%	25%	37.5%	16.65
LN	17	14	21	38	34	18	9	24	5
$n = 65$	30.9%*	25.5%*	38.2%*	58.5%*	52.3%	27.7%*	13.8%**	36.9%	7.7%*

SLE, systemic lupus erythematosus; ANA, anti-nuclear antibodies; anti-dsDNA, anti-double-stranded DNA; anti-cmDNA, anti-cell membrane DNA; NPSLE, neuropsychiatric lupus; LN, lupus nephritis. *$P < 0.05$, compared with NPSLE group; **$P < 0.01$, compared with NPSLE group. The χ^2 test was used to compare percentages.

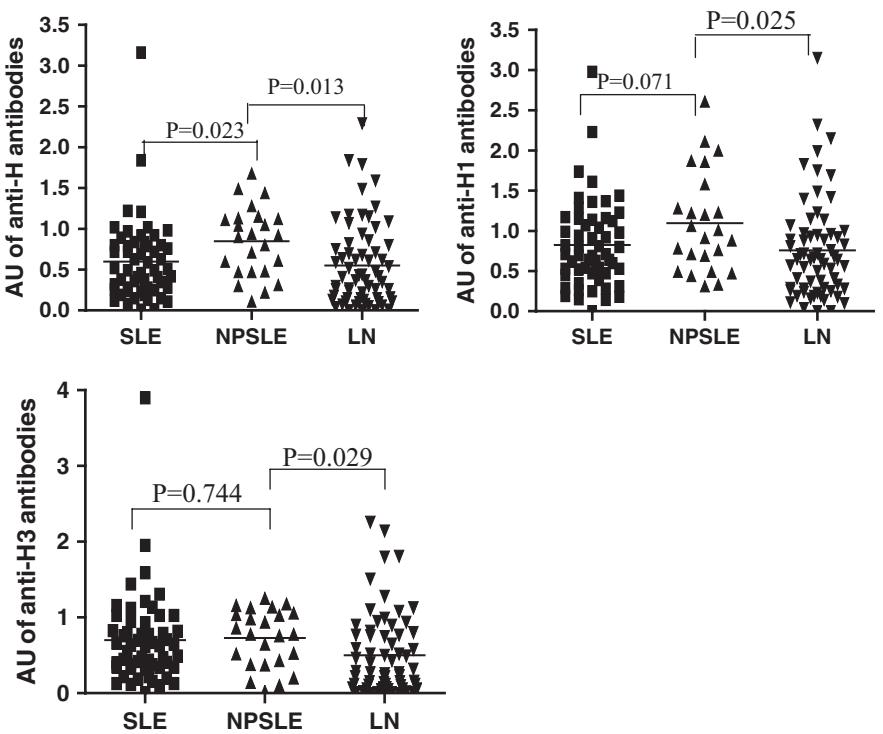

Fig. 1. Levels of antibody to H, H1, and H3 in different subgroups of SLE. SLE, systemic lupus erythematosus; NPSLE, neuropsychiatric lupus; LN, lupus nephritis; general SLE means patients without injury of kidney and neuropsychiatric.

other hand, antibodies to dsDNA, Sm, and DNP were detected in 37.5, 25, and 37.5% of the patients with NPSLE, which were much lower than antibodies to histones, H1, and H3, respectively. Thus, the tests distinguishing best between NPSLE and non-NPSLE patients were anti-H1 and anti-H ($P < 0.01$, Fisher's exact test).

Disease Activity in SLE Patients With and Without Autoantibodies to Histones or Others

Patients with an anti-H and anti-H1 antibody response showed significantly higher disease activity, as assessed by SLEDAI score, than patients without such response (mean SLEDAI, $P < 0.05$; Table 3). In addition, SLEDAI scores also significantly correlated with the presence of anti-dsDNA ($P = 0.04$) or AnuA ($P = 0.028$) antibodies. In contrast, no significant association with SLEDAI scores was found for other antibodies. SLEDAI scores of patients positive for each antibody did not significantly differ from each other, and the highest values were observed with anti-H1 followed by anti-H.

Statistical analyses using Spearman's correlation coefficient revealed a strong correlation of anti-H with scores of clinical disease activity (SLEDAI, $r = 0.18$, $P = 0.032$; Fig. 2A) as well as anti-H1 antibody ($r = 0.19$, $P = 0.024$; Fig. 2B).

TABLE 3. Association of SLEDAI Scores and Autoantibodies in SLE

	Antibodies		
Antigen	Positive SLEDAI±SD	Negative SLEDAI±SD	*P*-value
H	16.4±6.96	12.9±6.09	0.038
H1	16.5±6.88	13.1±6.24	0.037
H3	15.5±6.93	14.2±6.19	NS
ANA	14.9±6.47	13.3±6.03	NS
dsDNA	15.3±6.31	11.9±6.34	0.04
AnuA	15.3±6.06	11.4±6.33	0.029
cmDNA	14.6±6.6.2	14.3±6.29	NS
Sm	14.1±8.27	14.5±6.01	NS
DNP	15.6±6.12	14.4±6.52	NS

SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; ANA, anti-nuclear antibodies; dsDNA, double-stranded DNA; cmDNA, cell membrane DNA. Data are from 144 patients with SLE. Patients with positive and negative antibody reactivities to histone H, H1, H3, nuclear, dsDNA, Sm, cmDNA, nucleosome, and DNP are compared with respect to their SLEDAI scores. Note the significantly higher SLEDAI scores in patients positive for antibody responses against H, dsDNA, and nucleosome.

Associations Between Antibody to Histones and AnuA or dsDNA in SLE

In 144 SLE patients, the frequencies of antibody to H, H1, and H3 positive were similar to that of dsDNA positive (Table 4). There was a statistical correlation between anti-H (CO = 0.196, $P = 0.026$) or anti-H1 (CO = 0.266, $P = 0.003$) and dsDNA using Kendall's Turb test. In contrast, there was no correlation between anti-H3 and dsDNA. There was a significantly lower frequency of antibody positive between and dsDNA (McNemar test, $P = 0.023$). In addition, there was no significant difference of the frequency of antibody positive between anti-H or anti-H3 and dsDNA.

As far as AnuA antibody was concerned, its positive rate was strongly associated with the presence of antibodies to H and H1 (Table 5). At the same time, the frequency of AnuA positive was significantly higher than that of anti-H, anti-H1, or anti-H3 positive (McNemar test, $P < 0.01$; Table 5).

The Frequency of Antibodies to H, H1, and H3 in SLE Lacking DNP, cmDNA, and Sm Antibodies

The frequencies of antibodies to H, H1, and H3 in SLE lacking DNP as well as cmDNA and Sm antibodies were analyzed, respectively (Table 6). The results indicated that 24 (19.8%) and 33 (27.3%) patients with antibodies to H, H1, or H3 were found from 121 patients lacking anti-Sm antibodies, respectively. In the patients without anti-DNP, the prevalence of antibodies to H, H1, or H3 was somewhat higher than those patients without anti-Sm antibody, from 22.6% (29/128) and 32% (41/128), respectively. As for the patients without anti-cmDNA, there were about from 18.9%

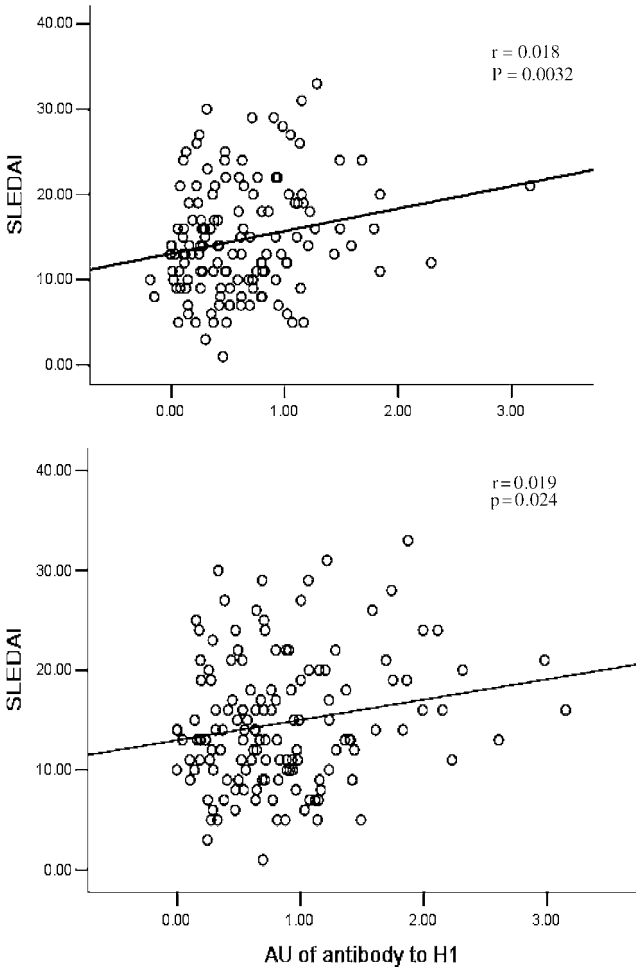

Fig. 2. Correlation of antibodies to H and H1 and SLEDAI scores. SLEDAI disease activity scores of SLE patients were correlated with antibody responses to histones (H), H1, and nucleosome ($r = 0.197$, $P = 0.026$ data were not shown). SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index.

TABLE 4. Cross Tabulation of the Frequencies of Antibody to Histones and dsDNA in SLE

		dsDNA		McNemar test	Kendall's Turb test	
Antibodies		Positive	Negative	*P*	CO	*P*
Anti-H	Positive	25	23	0.253	0.196	0.026
	Negative	26	69			
Anti-H1	Positive	15	22	0.023	0.266	0.003
	Negative	36	71			
Anti-H3	Positive	28	25	0.583	0.142	0.106
	Negative	23	68			

Data are shown as numbers. dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus.

TABLE 5. Cross Tabulation of the Frequencies of Antibody to Histones and AnuA in SLE

		AnuA		McNemar test	Kendall's Turb test	
Antibodies		Positive	Negative	*P*	CO	*P*
Anti-H	Positive	32	16	0.000	0.215	0.012
	Negative	44	52			
Anti-H1	Positive	26	11	0.000	0.252	0.003
	Negative	50	57			
Anti-H3	Positive	31	22	0.004	0.092	0.294
	Negative	45	46			

Data are shown as numbers. SLE, systemic lupus erythematosus.

(17/90) to 28.9% (27/90) patients who were anti-H, H1, and H3 positive.

DISCUSSION

The frequency of histone antibodies in SLE has been reported from 17 to 95% (average = 51%) (6,9,14,15,23,24,25). In our study, a cross-sectional analysis of approximately 300 sera of patients with various rheumatic autoimmune diseases and HCs revealed an average 33% sensitivity and high specificities (93.6 or 96.4%) of antibodies against histones or their subclass H1 and H3 for SLE. Moderate or high reactivities to histones, H1, and H3 were only found in patients with SLE. Low frequency of antibodies against histones has been described to occur in RA (26), juvenile RA (25), SSc (27), SS (28), and PM/DM (29). Our study showed that such low responses were very rare in non-SLE subjects: 24% (6/25) patients with SSc, 7% (1/14%) patients with PM/DM, and 7% (2/28) patients with RA, whereas none of the patients with SS and HCs had anti-histones antibody. The underlying molecular mechanism needs to be investigated in the future.

Previous studies on the association of anti-histone antibodies with disease activity are very discrepant. Some reports showed no association between presence or amount of anti-histone antibodies and any measure of disease activity (30–33) with the exception of history of photosensitivity in one report (33) and joint disease in another (29). On the other hand, associations of anti-histone antibodies with active disease were observed with solid-phase (34) and histone-reconstituted immunofluorescence assays (35). Population correlations of the presence of anti-histone antibodies with neuropsychiatric involvement (36), skin and joint symptoms (37), or overall disease severity have been reported (17). This study showed a strong correlation with SLE disease activity (SLEDAI) and levels of antibody to histones and H1. In addition, higher scores of SLEDAI were observed in patients with positive antibody than those in antibody negative group. The results agree with earlier observations (6,38,39).

Significantly, in the cross-sectional analysis, anti-H, anti-H1, and anti-H3 were significantly associated with NPSLE. Antibodies to histones, H1, and H3 testing showed average 50% sensitivity for NPSLE, which was higher than average 25.6% sensitivity for SLE and average 31.5% sensitivity for LN. In addition, the titer of antibodies against histones, H1, and H3 in patients with NPSLE was higher than that in patients with SLE and LN. The sequential analysis confirmed that the constellation of low disease activity and high anti-dsDNA was very uncommon in large mount of serum samples of patients with moderate or high disease activity; however, anti-dsDNA antibodies were not positive. In contrast, anti-H or H1 antibody levels were elevated in these samples. These results strongly suggest employing the assessment of anti-histones as a useful tool to monitor disease activity, particularly in patients lacking anti-dsDNA antibodies.

In summary, our study has shown that antibodies of anti-histones, anti-H1, and anti-H3 are highly specific markers for SLE (93.6–96.4%) and are closely associated with disease activity. The antibodies have a prevalence of about 50% in NPSLE. In addition, anti-histones ELISA is a reliable and easy assay that is particularly attractive for diagnostic purposes.

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