Anti-dsDNA and Antichromatin Antibody Isotypes in Serologically Active Clinically Quiescent Systemic Lupus Erythematosus

Amanda J. Steiman, Murray B. Urowitz, Dominique Ibañez, Timothy T. Li, Dafna D. Gladman, and Joan Wither

ABSTRACT. ***Objective.*** Serologically active clinically quiescent (SACQ) patients with systemic lupus erythematosus (SLE) are clinically quiescent despite serologic activity. Since studies suggest that antichromatin antibodies are more sensitive than anti-dsDNA antibodies in detecting active SLE, and that immunoglobulin (Ig) G, in particular complement-fixing subclasses, may be more pathogenic than IgM, we investigated the levels of anti-dsDNA and antichromatin isotypes in SACQ patients as compared to non-SACQ patients with SLE.

Methods. Levels of IgM, IgA, IgG, and IgG1–4 antichromatin and anti-dsDNA were measured by ELISA. SACQ was defined as ≥ 2 years with the SLE Disease Activity Index 2000 (SLEDAI-2K) at 2 or 4 from serologic activity, during which patients could be taking antimalarials, but not corticosteroids or immunosuppressives. Unselected non-SACQ patients with SLE were used as comparators. SACQ patient serum samples were further stratified based on subsequent development of flare, defined as clinical SLEDAI-2K ≥ 1 and/or treatment initiation. Nonparametric statistics were used, and generalized estimating equations were applied to account for multiple samples in the same patient.

Results. SACQ patients' complement-fixing antichromatin and anti–dsDNA IgG subclasses were significantly higher than those of non-SACQ patients. When the sample drawn latest in a SACQ period was analyzed, there was no difference between antichromatin or anti-dsDNA isotype or IgG subclass levels between patients who flared and those who remained SACQ, nor were consistent trends seen when samples were examined during SACQ and flare in the same patient.

Conclusion. The SACQ phenotype does not arise from a lack of pathogenic anti-dsDNA and/or antichromatin autoantibodies. Neither increases in antichromatin nor anti-dsDNA isotype or IgG subclass levels were predictive of or coincident with flare in SACQ patients. (First Release March 1 2015; J Rheumatol 2015;42:810–16; doi:10.3899/jrheum.140796)

Key Indexing Terms:
DISEASE ACTIVITY SEROLOGIC ACTIVITY SYSTEMIC LUPUS ERYTHEMATOSUS
OUTCOME ANTI-DNA ANTIBODIES ANTICHROMATIN ANTIBODIES

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by exacerbations and remissions in affected organ systems. In many patients with SLE, serum levels of anti-dsDNA antibodies and/or complement correlate with disease course, and can thus be of use in predicting disease flare; however, in other patients, clinical and serologic courses are discordant[1]. One such group of patients, termed serologically active clinically quiescent (SACQ), manifests persistent elevations in anti-dsDNA and/or hypocomplementemia in the

From the University of Toronto; Centre for Prognosis Studies in The Rheumatic Diseases, Toronto Western Hospital; Toronto Western Hospital; Arthritis Centre of Excellence, Division of Genetics and Development, Toronto Western Hospital Research Institute, University Health Network, Toronto, Ontario, Canada.

The University of Toronto Lupus Clinic is funded by the University Health Network, the Toronto General and Western Hospital Foundation, and the Arthritis Research Foundation. Dr. Steiman's work has been supported by the 2011 Arthritis Centre of Excellence fellowship, 2011–2012 Geoff Carr Fellowship, and 2012–2014 UCB-CRA-TAS Postgraduate Rheumatology Fellowship. Dr. Wither is supported by the Arthritis Centre of Excellence and the Arthritis and Autoimmunity Research Centre.

A.J. Steiman, MD, FRCPC, Rheumatology Fellow, University of Toronto, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; M.B. Urowitz, MD, FRCPC, Professor of Medicine, University of Toronto, and Director, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; D. Ibañez, MSc, Biostatistician, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; T.T. Li, MSc, Medical Student, Arthritis Centre of Excellence, Division of Genetics and Development, Western Hospital Research Institute, University Health Network; D.D. Gladman, MD, FRCPC, Professor of Medicine, University of Toronto, and Deputy Director, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; J. Wither, MD, PhD, FRCPC, Professor of Medicine, University of Toronto, Arthritis Centre of Excellence, Division of Genetics and Development, Toronto Western Hospital Research Institute, University Health Network.

Address correspondence to Dr. J. Wither, Toronto Western Hospital, 399 Bathurst St., 1E-420, Toronto, Ontario M5T 2S8, Canada. E-mail: jwither@uhnresearch.ca

Accepted for publication January 2, 2015.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

absence of clinical manifestations of SLE over a protracted period of time[2].

Currently, the mechanisms by which SACQ patients remain clinically inactive despite elevations of anti-dsDNA and/or hypocomplementemia remain unclear. It has been suggested that not all anti-dsDNA are equally pathogenic, with anti-dsDNA of high avidity, of immunoglobulin (Ig) G isotype, and complement-fixing IgG subclass best correlating with disease activity and renal involvement[3,4,5,6,7,8,9,10,11]. As a corollary, IgM isotype and non-complement-fixing IgG subclasses best correlate with disease quiescence. Further, antibodies to chromatin (antichromatin), the native nuclear form of DNA and histone proteins (or its repeating element, the nucleosome), have been shown by some to be of higher sensitivity and specificity than anti-dsDNA for SLE disease activity, especially when the latter is not present or proves discordant[12,13,14,15,16,17,18,19,20]. These findings raise the possibility that the anti-dsDNA and antichromatin Ig isotypes of SACQ patients are less pathogenic than those seen in the general SLE population.

In our study, we have examined anti-dsDNA and antichromatin Ig isotypes in SACQ and a cohort of unselected non-SACQ patients with SLE to address this possibility. We show that SACQ patients have at least equivalent levels of pathogenic Ig isotypes to other patients with SLE. We further demonstrate that there is no difference in Ig isotypes between SACQ patients who remain SACQ and those who ultimately flare, nor were consistent changes seen in the same patients assayed during the SACQ period and at flare. Thus, alternate immunologic mechanisms must be sought to explain SACQ patients' clinical quiescence.

MATERIALS AND METHODS

Patient selection. The University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases was established in 1970 to study clinical-laboratory correlations in SLE. All patients fulfilled 4 or more of the 1971 or 1982 American College of Rheumatology classification criteria, or 3 criteria together with a typical renal biopsy lesion of SLE. The clinic's patients range from those with acutely active disease to patients in complete remission and not receiving any therapy[21]. All patients were followed with clinical and laboratory information collected using a standardized protocol at clinic visits, typically at 2-month to 6-month intervals that occur regardless of disease activity. For our study, patients registered in the Lupus Clinic database between July 1970 and April 2008 with visits no more than 18 months apart were identified.

SACQ was defined as at least a 2-year period without clinical activity but with persistent serologic activity [SLE Disease Activity Index 2000 (SLEDAI-2K) = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit]. The patients could be taking antimalarials, but those treated with corticosteroids or immunosuppressive medications were excluded, ensuring the clinicians' impression of clinical quiescence. Disease flare was defined by any increase in the SLEDAI-2K at a clinic visit not accounted for by either hypocomplementemia or anti-dsDNA, or by the initiation of corticosteroid or immunosuppressive treatment. Unselected patients with SLE were used as controls and consisted of non-SACQ clinic patients with variable clinical and serologic activity whose serum was archived within 1 year of a corresponding SACQ patient. Healthy controls were recruited from hospital and laboratory staff members who had no family history of autoimmune disease. The mean age of the controls was 34.8 years and 87.5% were women. All subjects signed informed consent forms and patients signed a consent form that allowed their clinical, serologic, and genetic material to be studied and reported.

Laboratory analyses. Archived serum samples of patients stored at –80°C that had not undergone a previous freeze-thaw cycle were retrieved. Serum levels of IgM, IgA, IgG, IgG1, IgG2, IgG3, and IgG4 anti-dsDNA and antichromatin antibodies were measured by ELISA using an approach adapted from our previous mouse and human SLE studies[22,23]. Briefly, H1-stripped chromatin (mainly mono- and dinucleosomes) was prepared from the human cell line, MOLT4[24]. ELISA plates (Immunolon 2HB, VWR) were coated overnight with dsDNA (40 μg/ml) or chromatin (8 μg/ml) diluted in phosphate buffered saline at 4°C. Serum was diluted 1/100 for the measurement of IgM, IgA, and IgG, or 1/50 for IgG1–4. Bound antibodies were detected using alkaline-phosphatase conjugated goat anti-human IgM, IgA, or IgG antibodies (1:1000 dilution, all from Cedarlane), or biotin conjugated mouse anti-human IgG1–4 antibodies (1:500 dilution, all from Cedarlane except IgG2 from Caltag), followed by detection with alkaline-phosphatase conjugated streptavidin (1:1000 dilution). Absorbance was read at 405 nm. All assays were performed in triplicate. To control for interplate and interassay variability, the same positive control sera were on each plate. Although these sera were included to enable normalization of the sample values between plates if significant differences were noted, the values for these sera were very reproducible between assays and, therefore, no normalization was required between plates. To ensure that the antibodies being measured were specific for nucleosomes or dsDNA, plates with no antigen bound were included in every assay, and binding in the absence of antigen was subtracted.

Statistical analysis. Wilcoxon rank sum tests and generalized estimating equations (GEE) were used to report p values. The use of multiple samples from 1 patient was adjusted for through the use of a repeated measure model with an exchangeable correlation structure.

RESULTS

Thirty-eight serum samples from 23 patients, drawn during a prolonged SACQ period, were analyzed. Fifteen of the 38 samples (39%) corresponded to 9 patients whose SACQ period eventually ended in flare. Patient demographics did not differ between those who remained SACQ and those who ultimately flared (Table 1). Among those who flared, disease activity manifested mainly as arthritis or mucocutaneous involvement; 1 patient had serositis, another hemolytic anemia necessitating corticosteroid use, and none had major organ manifestations. Twenty-one unselected non-SACQ patients with SLE, whose serum was stored contemporaneously, were used as comparators. These patients had an age and disease duration similar to those of the SACQ patients, but were more likely to have received steroids and/or immunosuppressive medications than the SACQ patients. Similar proportions of SACQ patients and non-SACQ patients were treated with antimalarials at the time of study, but 71% of the non-SACQ patients were also receiving prednisone and 67% were taking immunosuppressive medications. The majority of patients were clinically active [17/21 (81%), clinical SLEDAI > 0] with a mean SLEDAI of 7.9 ± 5.9. All 23 SACQ patients and 20/21 of non-SACQ patients (95.2%) were anti-dsDNA–positive

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

Table 1. Patient demographics divided by SACQ outcome. Values are mean ± SD or n (%) unless otherwise specified.

Characteristics	Non-SACQ Patients with SLE, n = 21	SACQ Patients Flare, n = 9	SACQ Patients Quiescent, n = 14	p Between SACQ Subsets
Female	20 (95)	9 (100)	11 (78.6)	0.25
Age at sample date, yrs	48.5 ± 13.0	43.7 ± 15.3	47.7 ± 20.5	0.73
Disease duration at sample date, yrs	20.5 ± 9.9	20.1 ± 14.4	13.7 ± 9.3	0.37
Steroids ever	15 (71.4)	5 (55.6)	9 (64.3)	1.00
Antimalarials ever	13 (61.9)	8 (88.9)	11 (78.6)	1.00
Immunosuppressives ever	14 (66.7)	1 (11.1)	2 (14.3)	1.00
Steroids current	15 (71.4)	N/A	N/A	N/A
Steroids dose, mg	16.8 ± 14.5	N/A	N/A	N/A
Antimalarials current	13 (61.9)	6 (66.7)	7 (50.0)	0.67
Immunosuppressives current	14 (66.7)	N/A	N/A	N/A

SACQ: serologically active clinically quiescent; SLE: systemic lupus erythematosus; N/A: not applicable.

(by Farr assay, p = 0.48), with mean levels 25.0 ± 22.9 u/ml and 44.9 ± 33.0 u/ml, respectively (p = 0.03).

With the exception of IgM anti-dsDNA antibodies (which were lower in non-SACQ patients with SLE), antichromatin and anti-dsDNA IgG, IgM, IgG, or IgG/IgM levels among SACQ patients (using only the most recent sample when more than 1 sample was available) were not significantly different from those seen in the non-SACQ SLE cohort, and were significantly elevated as compared to healthy controls (Table 2). In general, the proportion of SACQ and non-SACQ patients with SLE who had elevated levels (> 2 SD above mean) of IgA, IgM, or IgG anti-dsDNA or antichromatin antibodies also did not differ, except that SACQ patients had an increased proportion of IgM anti-dsDNA antibodies [anti-dsDNA SACQ vs non-SACQ: IgA 13% vs 14%, IgM 35% vs 0% (p = 0.004), IgG 65% vs 38%. Antichromatin SACQ vs non-SACQ: IgA 43% vs 38%, IgM 48% vs 38%, IgG 91% vs 67%]. Further, when subdivided by IgG subclass (and after adjusting for multiple comparisons), SACQ patients' levels of antichromatin IgG1 and 3, and anti-dsDNA IgG1 were significantly higher than those of the non-SACQ patients with SLE, with a trend toward higher levels of IgG2 in SACQ patients for both autoantibodies. IgG4 did not differ between groups (Figure 1).

When a single sample drawn during a SACQ period per patient was analyzed, there was no difference between antichromatin or anti-dsDNA isotypes, or IgG subclass levels between SACQ patients who flared and those who remained SACQ (Table 3). When all samples were included using a GEE, antichromatin IgG2 and anti-dsDNA total IgG levels were significantly higher in SACQ patients who remained quiescent (p < 0.0001 for both, data not shown). There was no difference in antichromatin or anti-dsDNA IgG/IgM ratio in those SACQ patients who ultimately flared versus those who did not (Table 3). There was no correlation between the levels of anti-dsDNA or antichromatin Ig

Table 2. Antichromatin and anti-dsDNA levels in SACQ patients as compared to unselected non-SACQ patients and healthy controls. Values are mean ± SD (range) unless otherwise specified.

Characteristics	SACQ[1], n = 23	Non-SACQ Patients, n = 21	Healthy Controls, n = 49	p SACQ vs Healthy Controls	p Non-SACQ vs Healthy Controls
Antichromatin					
IgA	0.07 ± 0.10 (0–0.37)	0.04 ± 0.04 (0–0.14)	0.01 ± 0.02 (0–0.07)	< 0.0001	< 0.0001
IgM	0.52 ± 0.67 (0–2.18)	0.39 ± 0.53 (0–1.76)	0.06 ± 0.08 (0–0.32)	< 0.0001	0.0002
IgG	0.59 ± 0.57 (0.07–2.21)	0.40 ± 0.39 (0.04–1.44)	0.08 ± 0.04 (0.01–0.20)	< 0.0001	< 0.0001
IgG/IgM[2]	26.6 ± 80.3 (0.03–317)	16.2 ± 45.3 (0–163)	0.53 ± 0.31 (0.14–1.26)	0.75	0.29
Anti-dsDNA					
IgA	0.06 ± 0.07 (0–0.28)	0.05 ± 0.04 (0.02–0.15)	0.02 ± 0.04 (0–0.22)	0.0004	< 0.0001
IgM	0.38 ± 0.26 (0–0.94)	0.13 ± 0.07 (0–0.30)	0.23 ± 0.10 (0.07–0.49)	0.0030	< 0.0001
IgG	0.40 ± 0.62 (0.03–3.04)	0.22 ± 0.19 (0–0.66)	0.10 ± 0.04 (0.04–0.28)	< 0.0001	0.0011
IgG/IgM	81.2 ± 183 (0.01–774)	15.3 ± 53.2 (0.30–246)	26.3 ± 39.8 (0.03–197)	0.012	< 0.0001

[1] There were no significant differences between SACQ and non-SACQ patients with SLE. [2] IgM values of 0 were changed to 0.001, the minimum detectable limit of the ELISA, to enable calculation of an IgG/IgM ratio for all participants. SACQ: serologically active clinically quiescent; Ig: immunoglobulin; SLE: systemic lupus erythematosus.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

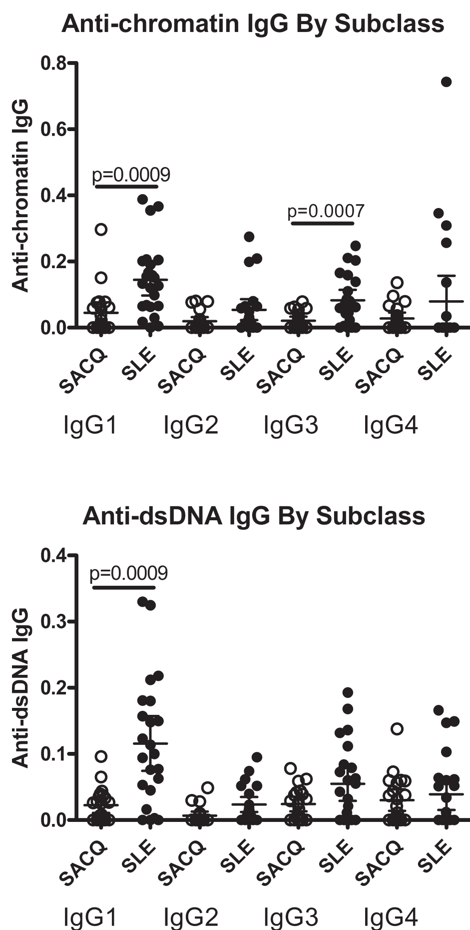

Figure 1. Comparison of IgG subclass, measured by ELISA, between SACQ patients with SLE and unselected SLE controls. IgG: immunoglobulin G; SACQ: serologically active clinically quiescent; SLE: systemic lupus erythematosus.

isotype or IgG subclass and time to flare. The mean time to flare (or most recent SACQ visit) from last sample analyzed was 2.1 years and 2.2 years in SACQ patients who remained SACQ and those who flared, respectively (ranges 1.0–3.8 yrs and 0.7–6.9 yrs, respectively). Following adjustment for the length of time between sample outcome dates, there was still no correlation between the levels of autoantibodies and flare (data not shown). A time-to-event analysis corroborated these negative findings.

There were only 5 patients in whom serologic samples were available both during SACQ periods and at the time of flare. Given the small sample size, these data were analyzed only qualitatively. (In the cases where > 1 sample was available for a disease state, the mean autoantibody level was recorded.) There did not appear to be any trend in fluctuations in autoantibody levels between SACQ and flare in any of these patients (Table 4).

DISCUSSION

SACQ patients with SLE represent a unique phenotype with clinical and serologic discordance. Previously, we have shown that among this unique patient subset, reflecting 6% of our SLE cohort, there are no clinical predictors of flare. Similarly, fluctuations in anti-dsDNA and/or complement levels in SACQ patients during a SACQ period were not predictive of disease flare[25]. The observation that SLE-related organ damage does not accrue subclinically during a prolonged SACQ period supports the practice of close clinical monitoring without the use of prophylactic corticosteroid or immunosuppressive medications[26]. However, damage does begin to accrue again once these patients flare, and thus a serologic marker of impending flare would permit early reintroduction of therapy. In our study, we examined autoantibody isotypes and IgG subclasses in SACQ patients to determine whether the lack of clinical symptoms in these patients arises from the presence of less pathogenic autoantibodies and to address whether changes in the level and types of autoantibodies predict or occur concurrently with subsequent flares.

Previously, increased levels of IgG as compared to IgM anti-dsDNA antibodies have been shown to be associated with active disease. For instance, Kessel, *et al* found that the SLEDAI was increased in patients whose anti-dsDNA IgG titers were higher than anti-dsDNA IgM titers[9]. Förger, *et al* also studied the anti-dsDNA IgG/IgM ratio in over 200 patients with SLE, and found that this ratio was a significant variable in distinguishing patients with and without nephritis[7]. Similar findings were observed by Villalta, *et al*, who also observed an association between anti-dsDNA IgA and IgA/IgM ratio with renal disease in SLE[27]. It has been theorized that IgM may be protective because of its ability to downregulate autoreactive B cells, resulting in decreased pathogenic IgG production[9]. Alternatively, IgM may act by binding circulating antigens, activating complement, and accelerating immune complex clearance. In support of this concept, administration of anti-dsDNA IgM to SLE-prone mice resulted in attenuated renal pathology and improved survival[28]. In contrast to these previous studies, we found no significant difference between the levels of IgG, IgM, or IgG/IgM ratio between SACQ and non-SACQ patients, and a trend toward somewhat higher IgG and lower IgM in SACQ patients who remained SACQ compared to those who flared (Table 2). While these later results did not achieve statistical significance (except for IgG anti-dsDNA using the GEE model), the consistency of this trend across both antichromatin and anti-dsDNA, regardless of whether 1 or multiple samples were analyzed, suggests that "protective" serology is not the driver of SACQ status.

Antinucleosome antibodies are among the first detected in the sera of patients with SLE. They are thought to be pathogenic, as evidenced by their presence in glomerular deposits and eluates of patients with SLE nephritis[19,29,30]. In

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

Table 3. Mean antichromatin and anti-dsDNA levels in SACQ patients who subsequently flared versus those who remained SACQ (1 sample per patient). Values are mean ± SD unless otherwise specified.

Characteristics	Antichromatin			Anti-dsDNA		
	Flare, n = 9	SACQ, n = 14	p	Flare, n = 9	SACQ, n = 14	p
IgA	0.077 ± 0.117	0.071 ± 0.085	0.93	0.032 ± 0.030	0.073 ± 0.087	0.34
IgM	0.664 ± 0.909	0.430 ± 0.480	0.83	0.434 ± 0.220	0.338 ± 0.287	0.12
IgG	0.423 ± 0.314	0.691 ± 0.682	0.60	0.297 ± 0.139	0.474 ± 0.788	0.48
IgG1	0.133 ± 0.112	0.153 ± 0.112	0.60	0.078 ± 0.065	0.141 ± 0.106	0.17
IgG2	0.031 ± 0.026	0.069 ± 0.089	0.56	0.026 ± 0.033	0.022 ± 0.023	0.78
IgG3	0.095 ± 0.080	0.075 ± 0.070	0.60	0.049 ± 0.054	0.058 ± 0.060	0.85
IgG4	0.132 ± 0.253	0.046 ± 0.110	0.50	0.046 ± 0.058	0.035 ± 0.054	0.66
IgG/IgM	1.26 ± 1.66	1.79 ± 2.87	0.60	1.46 ± 0.97	3.76 ± 7.73	1.00

SACQ: serologically active clinically quiescent; Ig: immunoglobulin.

Table 4. Comparison of autoantibody levels taken in the same patient during SACQ versus during flare*. Values are mean optical density as determined by ELISA.

Characteristics	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	SACQ	Flare	SACQ	Flare	SACQ	Flare	SACQ	Flare	SACQ	Flare
Antichromatin										
IgA	0.000	0.110	0.004	0.046	0.149	**0.130**	0.066	**0.058**	0.018	0.059
IgM	0.000	0.314	0.155	**0.000**	0.000	0.198	0.117	0.350	0.048	0.254
IgG	0.308	1.474	0.561	**0.279**	0.774	**0.723**	0.602	0.709	1.237	**1.094**
IgG1	0.152	**0.146**	0.108	**0.006**	0.206	0.320	0.202	**0.182**	0.117	0.180
IgG2	0.016	0.212	0.052	**0.000**	0.080	**0.054**	0.064	0.102	0.110	0.274
IgG3	0.087	0.128	0.037	0.090	0.005	0.180	0.159	0.169	0.108	0.161
IgG4	0.000	0.316	0.000	0.058	0.000	0.292	0.000	0.142	0.000	0.040
Anti-dsDNA										
IgA	0.004	0.065	0.055	**0.046**	0.022	0.058	0.030	0.048	0.044	0.083
IgM	0.421	0.436	0.264	**0.224**	0.000	0.139	0.762	**0.502**	0.084	0.230
IgG	0.206	0.613	0.185	**0.151**	0.317	**0.264**	0.536	**0.201**	0.357	0.750
IgG1	0.045	0.125	0.096	**0.048**	0.180	**0.041**	0.100	**0.068**	0.120	**0.070**
IgG2	0.014	0.032	0.008	**0.000**	0.000	**0.000**	0.517	**0.000**	0.026	**0.007**
IgG3	0.060	**0.010**	0.057	**0.000**	0.000	0.022	0.132	**0.000**	0.113	**0.016**
IgG4	0.060	**0.038**	0.008	0.066	0.147	**0.000**	0.000	0.095	0.081	**0.043**

* Bold face data indicate decreased level in flare state. SACQ: serologically active clinically quiescent; Ig: immunoglobulin.

fact, it is only through epitope spreading that nucleosome-specific T cells stimulate B cells to produce anti-dsDNA and antihistone antibodies[19]. In contrast, although anti-dsDNA is a hallmark of SLE, free dsDNA has been found to be poorly immunogenic. In their native state, strands of dsDNA are complex with histones in chromatin, and chromatin rather than free dsDNA is thought to be one of the main drivers of the immune response in SLE[31]. Consistent with this central role of chromatin, there is general consensus that antinucleosome antibodies are both sensitive and specific for SLE diagnosis and disease activity[12,19,20,32], although the antinucleosome antibodies have also been reported in the antiphospholipid syndrome[33]. A metaanalysis by Bizzaro, *et al* determined that in selected studies, antinucleosome antibodies, but not anti-dsDNA, were associated with disease activity ($p < 0.0001$)[19]. This is further supported by a study by Suleiman, *et al*, who found that antinucleosome antibodies were 98% sensitive and 86% specific for detecting active SLE, versus 61% and 84% for anti-dsDNA sensitivity and specificity, respectively[12]. On the basis of these findings, we reasoned that the levels of antichromatin antibodies would correlate more closely than anti-dsDNA with the difference in disease activity between SACQ and non-SACQ patients, or with flares in disease activity within the SACQ population. However, our results for antichromatin antibodies were remarkably similar to those for anti-dsDNA, indicating that clinical quiescence in SACQ patients does not arise from less pathogenic antichromatin isotypes. Further, they do not predict or occur concurrently with flares in these patients. Our findings contrast with those of Ng, *et al*[34], who investigated the levels of antinucleosome antibodies in "SACQ" patients and found that the presence and titer of these antibodies was significantly correlated with time to first flare after a SACQ

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

period. However, it should be noted that in their study, "SACQ" status was defined quite differently from ours as a British Isles Lupus Assessment Group index score < 6 without any mention of a requirement of successful weaning from corticosteroids and/or immunosuppressive medications. Thus, our patients represent a subset different from those studied by Ng, *et al*, whose patients had low — but not absent — disease activity that may have merely been suppressed by ongoing treatment rather than reflecting a true SACQ remission, as we had defined it.

There are significant differences in the function of the different subclasses of IgG. IgG1 and IgG3 are excellent activators of complement and bind efficiently to Fc receptors on proinflammatory cells, whereas IgG2 and IgG4 do not. Thus, differences in the subclasses of autoantibodies produced could affect clinical disease activity and tissue damage. Consistent with this concept, several studies have found that higher levels of IgG (over IgM) autoantibodies, especially of the complement-fixing subclasses IgG1 and IgG3, are predictive of SLE disease activity[5,9,28,35]. These findings raised the possibility that SACQ patients might maintain clinical quiescence through the production of less pathogenic IgG subclasses. However, this was not the case. Indeed, the levels of SACQ patients' complement-fixing antichromatin and anti-dsDNA IgG subclass autoantibodies were consistently significantly higher than those seen in the (generally clinically active) non-SACQ patients. Further, elevations in IgG1 or IgG3 were not seen in those SACQ patients who ultimately flared as compared to those who remained quiescent even when adjusted for time to event. While it could be argued that the significant latency between sample collection and time to flare in some patients prevented identification of trends that occurred immediately before the flare, the lack of consistent elevations in IgG1 and IgG3 autoantibodies in the 5 patients examined at the time of their flare argues against this point.

Our study suggests that SACQ patients remain clinically quiescent despite the presence of pathogenic autoantibodies. While the presence of relevant autoantigens is required for these antibodies to form immune complexes and mediate their damage, it appears that these autoantigens are not lacking in SACQ patients because the majority of SACQ patients are hypocomplementemic[25]. Thus, the findings in these patients diverge from the classic SLE paradigm that equates the presence of immune complexes with disease activity. Currently, the mechanism(s) that lead to this clinical quiescence remain to be fully determined. However, our ongoing studies suggest that proinflammatory factors are not produced in response to these complexes in SACQ patients.

In our small pilot study, we show that predicting clinical outcomes by serologic changes remains an elusive goal among SACQ patients. While our study was not powered to detect small differences between groups, the nature of the trends observed in our study suggest that even a sufficiently powered study would be unlikely to yield positive findings, and consequently, alternate biomarkers must be sought to aid in clinical decision making.

REFERENCES

1. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology 2007;46:1052-6.
2. Gladman DD, Urowitz MB, Keystone EC. Serologically active clinically quiescent systemic lupus erythematosus: a discordance between clinical and serologic features. Am J Med 1979;66:210-5.
3. Rothfield NF, Stollar BD. The relation of immunoglobulin class, pattern of anti-nuclear antibody, and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus. J Clin Invest 1967;46:1785-94.
4. Winfield JB, Faiferman I, Koffler D. Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity antinative DNA antibody with glomerulonephritis. J Clin Invest 1977;59:90-6.
5. Puritz EM, Yount WJ, Newell M, Utsinger PD. Immunoglobulin classes and IgG subclasses of human antinuclear antibodies. A correlation of complement fixation and the nephritis of systemic lupus erythematosus. Clin Immunol Immunopathol 1973;2:98-113.
6. Villalta D, Romelli PB, Savina C, Bizzaro N, Tozzoli R, Tonutti E, et al. Anti-dsDNA antibody avidity determination by a simple reliable ELISA method for SLE diagnosis and monitoring. Lupus 2003;12:31-6.
7. Förger F, Matthias T, Oppermann M, Becker H, Helmke K. Clinical significance of anti-dsDNA antibody isotypes: IgG/IgM ratio of anti-dsDNA antibodies as a prognostic marker for lupus nephritis. Lupus 2004;13:36-44.
8. Krippner H, Merle S, Jörgens K, Pirlet K. Antibodies to dsDNA and ssDNA in the immunoglobulin classes IgG and IgM: prognostic value in the course of SLE. Z Rheumatol 1984; 43:265-71.
9. Kessel A, Rosner I, Halasz K, Grushko G, Shoenfeld Y, Paran D, et al. Antibody clustering helps refine lupus prognosis. Semin Arthritis Rheum 2009;39:66-70.
10. Bijl M, Dijstelbloem HM, Oost WW, Bootsma H, Derksen RH, Aten J, et al. IgG subclass distribution of autoantibodies differs between renal and extra-renal relapses in patients with systemic lupus erythematosus. Rheumatology 2002;41:62-7.
11. Hahn BH. Antibodies to DNA. N Engl J Med 1998;338:1359-68.
12. Suleiman S, Kamaliah D, Nadeem A, Naing NN, Che Maraina CH. Anti-nucleosome antibodies as a disease activity marker in patients with systemic lupus erythematosus. Int J Rheum Dis 2009; 12:100-6.
13. Min DJ, Kim SJ, Park SH, Seo YI, Kang HJ, Kim WU, et al. Anti-nucleosome antibody: significance in lupus patients lacking anti-double-stranded DNA antibody. Clin Exp Rheumatol 2002;20:13-8.
14. Manson JJ, Ma A, Rogers P, Mason LJ, Berden JH, van der Vlag J, et al. Relationship between anti-dsDNA, anti-nucleosome and anti-alpha-actinin antibodies and markers of renal disease in patients with lupus nephritis: a prospective longitudinal study. Arthritis Res Ther 2009;11:R154.
15. Kiss E, Lakos G, Szegedi G, Poor G, Szodoray P. Anti-nuscleosome antibody, a reliable indicator for lupus nephritis. Autoimmunity 2009;42:393-8.
16. Gutiérrez-Adrianzén OA, Koutouzov S, Mota RM, das Chagas Medeiros MM, Bach JF, de Holanda Campos H. Diagnostic value of anti-nucleosome antibodies in the assessment of disease activity

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

of systemic lupus erythematosus: a prospective study comparing anti-nucleosome with anti-dsDNA antibodies. J Rheumatol 2006;33:1538-44.
17. Gómez-Puerta JA, Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) antibodies: diagnostic and clinical value. Autoimmunity Rev 2008;7:606-11.
18. Ghillani-Dalbin P, Amoura Z, Cacoub P, Charuel JL, Diemert MC, Piette JC, et al. Testing for anti-nucleosome antibodies in daily practice: a monocentric evaluation in 1696 patients. Lupus 2003;12:833-7.
19. Bizzaro N, Villalta D, Giavarina D, Tozzoli R. Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis. Autoimmun Rev 2012;12:97-106.
20. Heidenreich U, Mayer G, Herold M, Klotz W, Stempfl Al-Jazrawi K, Lhotta K. Sensitivity and specificity of autoantibody tests in the differential diagnosis of lupus nephritis. Lupus 2009;18:1276-80.
21. Urowitz MB, Gladman DD. Contributions of observational cohort studies in systemic lupus erythematosus: the University of Toronto lupus clinic experience. Rheum Dis Clin North Am 2005;31:211-21.
22. Cheung YH, Landolt-Marticorena C, Lajoie G, Wither JE. The lupus phenotype in B6.NZBc1 congenic mice reflects interactions between multiple susceptibility loci and a suppressor locus. Genes Immun 2011;12:251-62.
23. Li T, Prokopec SD, Morrison S, Lou W, Reich H, Gladman D, et al. Anti-nucleosome antibodies outperform traditional biomarkers as longitudinal indicators of disease activity in systemic lupus erythematosus. Rheumatology 2014 Sep 5 (E-pub ahead of print).
24. Yager TD, McMurray CT, Van Holde KE. Salt-induced release of DNA from nucleosome core particles. Biochemistry 1989; 28:2271-81.
25. Steiman AJ, Gladman DD, Ibañez D, Urowitz MB. Prolonged serologically active clinically quiescent systemic lupus erythematosus: frequency and outcome. J Rheumatol 2010;37:1822-7.
26. Steiman AJ, Gladman DD, Ibañez D, Urowitz MB. Outcomes in patients with systemic lupus erythematosus with and without a prolonged serologically active clinically quiescent period. Arthritis Care Res 2012;64:511-8.
27. Villalta D, Bizzaro N, Bassi N, Zen M, Gatto M, Ghirardello A, et al. Anti-dsDNA antibody isotypes in systemic lupus erythematosus: IgA in addition to IgG anti-dsDNA help to identify glomerulonephritis and active disease. PloS One 2013;8: e71458.
28. Werwitzke S, Trick D, Kamino K, Matthias T, Kniesch K, Schlegelberger B, et al. Inhibition of lupus disease by anti-double-stranded DNA antibodies of the IgM isotype in the (NZB x NZW)F1 mouse. Arthritis Rheum 2005;52:3629-38.
29. Muller S, Dieker J, Tincani A, Meroni PL. Pathogenic anti-nucleosome antibodies. Lupus 2008;17:431-6.
30. van der Vlag J, Berden JH. Lupus nephritis: role of antinucleosome autoantibodies. Semin Nephrol 2011;31:376-89.
31. Dieker JW, van der Vlag J, Berden JH. Triggers for anti-chromatin autoantibody production in SLE. Lupus 2002;11:856-64.
32. Pradhan VD, Patwardhan MM, Ghosh K. Anti-nucleosome antibodies as a disease marker in systemic lupus erythematosus and its correlation with disease activity and other autoantibodies. Indian J Dermatol Venereol Leprol 2010;76:145-9.
33. Andreoli L, Pregnolato F, Burlingame RW, Allegri F, Rizzini S, Fanelli V, et al. Antinucleosome antibodies in primary antiphospholipid syndrome: a hint at systemic autoimmunity? J Autoimmun 2008;30:51-7.
34. Ng KP, Manson JJ, Rahman A, Isenberg DA. Association of antinucleosome antibodies with disease flare in serologically active clinically quiescent patients with systemic lupus erythematosus. Arthritis Rheum 2006;55:900-4.
35. ter Borg EJ, Horst G, Hummel E, Limburg PC, Kallenberg CG. Rises in anti-double stranded DNA antibody levels prior to exacerbations of systemic lupus erythematosus are not merely due to polyclonal B cell activation. Clin Immunol Immunopathol 1991;59:117-28.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2015. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org