Lack of Interferon and Proinflammatory Cyto/chemokines in Serologically Active Clinically Quiescent Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Serologically active clinically quiescent (SACQ) patients with systemic lupus erythematosus (SLE) remain clinically quiescent for prolonged periods despite anti-dsDNA antibodies and/or low complements, indicating the presence of immune complexes. The immune mechanisms leading to this quiescence are unknown. However, in addition to activating complement, immune complex uptake by various cells leads to the production of interferon (IFN)-α and other proinflammatory factors that are also involved in tissue damage. Here we investigate whether production of these factors is reduced in SACQ patients.

Methods. The levels of 5 IFN-induced genes and 19 cyto/chemokines were measured in SACQ patients and were compared with those in serologically and clinically active (SACA) and serologically and clinically quiescent (SQCQ) patients. SACQ and SQCQ were defined as ≥ 2 years without clinical activity, with/without persistent serologic activity, respectively, and off corticosteroids/immunosuppressives. SACA was defined as disease activity compelling immunosuppression. Levels of *OAS1*, *IFIT1*, *MX1*, *LY6E*, and *ISG15* were measured by quantitative real-time polymerase chain reaction (PCR) and a composite score (IFN-5) derived from this. Plasma cyto/chemokines were measured by Luminex assay. Nonparametric univariate and logistic regression analyses were conducted.

Results. There were no differences in gene expression or cyto/chemokine levels between SACQ and SQCQ patients. The SACQ IFN-5 score was significantly lower than that of SACA ($p = 0.003$) and was driven by SACQ status, not by autoantibody profile or disease duration. Levels of granulocyte-macrophage colony-stimulating factor, interleukin (IL) 6, IL-10, IFN-γ-inducible protein 10, monocyte chemoattractant protein 1, and tumor necrosis factor-α were significantly lower in SACQ than SACA.

Conclusion. The levels of proinflammatory factors in SACQ mirror those of SQCQ patients, indicating reduced production of these factors despite the presence of immune complexes. (First Release November 15 2015; J Rheumatol 2015;42:2318–26; doi:10.3899/jrheum.150040)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS INTERFERON CYTOKINE CHEMOKINE

Elevations in anti-dsDNA antibodies and/or hypocomplementemia are often closely correlated with systemic lupus erythematosus (SLE) disease activity and are consequently included as the serologic components of the SLE Disease

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Activity Index 2000 (SLEDAI-2K), a well-validated tool for monitoring disease activity over time[1]. However, a unique subset of patients with SLE evolves from active disease to durable clinical quiescence despite the presence of persistent elevations in anti-dsDNA antibodies and/or hypocomplementemia[2]. This phenotype, termed "serologically active clinically quiescent" (SACQ), which conservatively represents 6% of patients with SLE in 1 cohort studied[3], is remarkable for its discordance between its pathogenic serology and concurrently benign clinical picture. Although ~60% of SACQ patients ultimately flare, they do so with relatively mild manifestations after a mean of 3 years of disease quiescence off corticosteroids and immunosuppressive medications. Patients who evolve to SACQ may also have a somewhat attenuated disease course. At initial presentation, they are less active and are receiving lower doses of corticosteroids than patients who do not evolve to SACQ, and while they have similar organ involvement at presentation and are just as likely to have renal disease or vasculitis over their disease course as non-SACQ patients, they are less likely to have musculoskeletal, skin, or central nervous system involvement[3]. SACQ patients also have less disease-associated and treatment-attributable organ damage, particularly after onset of the SACQ period[4].

Since the establishment of prolonged quiescence in the absence of treatment side-effects has been an elusive goal of clinicians treating SLE, a better understanding of the biologic processes leading to development of SACQ could shed light onto how best to achieve this goal. To date, the immunologic mechanisms that promote prolonged disease quiescence are unknown. In previous work, we have shown that SACQ status does not appear to arise from a lack of autoantibodies or immune complexes because the serum complements are low in many of these patients[3,5]. Further, SACQ patients do not differ from non-SACQ SLE controls on the basis of the levels of anti-dsDNA or antichromatin antibody isotypes or complement-fixing subclasses[5].

In SLE, immune complexes not only activate complement, but are also taken up by various immune populations leading to secretion of a wide range of proinflammatory factors. Of these, the Type I interferons (IFN), and particularly IFN-α, appear to be involved in disease pathogenesis. Patients with SLE have elevated levels of IFN-induced gene expression (the so-called "interferon signature"), which correlate with the presence of anti-dsDNA-associated and anti–RNA–associated autoantibodies, as well as disease activity[6–15,16,17,18]. Immune complexes from patients with SLE have been shown to induce plasmacytoid dendritic cells (pDC) to secrete large amounts of IFN-α[19,20,21,22], which has pleomorphic effects on the immune system, many of which exacerbate the autoimmune process[19,22]. For example, Type I IFN stimulates secretion of a variety of cyto/chemokines that augment inflammation, induces differentiation of monocytes to a mature myeloid DC phenotype that can better activate proinflammatory T cell subsets, and promotes B cell activation. Notably, elevations in cyto/chemokines that are induced directly by immune complexes [e.g., tumor necrosis factor-α (TNF-α) and interleukin (IL) 10] or augmented by Type I IFN [e.g. IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein (MCP) 1] have been shown to be associated with SLE disease activity in cross-sectional studies and thus have been proposed as potential biomarkers for this classically relapsing-remitting disease[12,23,24,25,26,27,28]. Given the association between elevations in IFN-induced gene expression and proinflammatory cyto/chemokines and SLE disease activity, we hypothesized that clinical quiescence in SACQ patients results from a lack of production of these proinflammatory cytokines, despite the presence of autoantibodies.

MATERIALS AND METHODS

Patients. Patients were recruited from the University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital. This clinic follows patients with SLE, typically at 2- to 6-month intervals regardless of disease activity, with clinical and laboratory information being collected using a standardized protocol. Patients registered in the clinic database between July 1970 and January 2012 with visits no more than 18 months apart were identified. All patients fulfilled 4 or more of the 1971 or 1982 American College of Rheumatology (ACR) classification criteria, or had 3 criteria with a typical renal biopsy lesion of SLE. SACQ was defined as at least a 2-year period without clinical activity and with persistent serologic activity (SLEDAI-2K = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit). Serologically quiescent clinically quiescent (SQCQ) patients were used as inactive controls and had at least a 2-year period with no clinical and serologic activity (SLEDAI-2K score = 0). Both SACQ and SQCQ patients could be taking antimalarials, but not corticosteroids or immunosuppressive medications. Peripheral blood for isolation of serum and RNA was drawn at the time of a regular followup clinic visit for SACQ and SQCQ patients, and thus the duration of clinical quiescence varied. Serologically active clinically active (SACA) patients were defined as those with disease activity by the SLEDAI-2K criteria, requiring the use of corticosteroids and/or immunosuppressive medications, and had their blood drawn at the time of clinical flare. All patients signed informed consents to allow their clinical, serologic, and genetic material to be studied and reported.

Gene expression analyses. Total RNA was prepared from peripheral blood preserved in PAXgene tubes (QIAGEN; obtained at the same visit as the patient's serum sample), as described[11], and converted to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time PCR amplification was performed using TaqMan primers for 5 IFN-induced genes: Hs00356631_g1 (*IFIT1*), Hs00182073_m1 (*MX1*), Hs00192713_m1 (*ISG15*), Hs00158942_m1 (*LY6E*), and Hs00242943_m1 (*OAS1*; Applied Biosystems). Normalization and quantification of the PCR signals was performed by comparing the cycle threshold value of the gene of interest with *GAPDH*. An IFN score, reflecting the sum of these 5 genes (IFN-5), was derived for each patient (similar to composite scores used in other studies)[10,11].

Cyto/chemokine analyses. Plasma cytokine and chemokine concentrations were measured using a 65-plex Luminex Cytokine panel (Eve Technologies) with all samples being run on the same assay. Nineteen cytokines were selected *a priori* for analysis: granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12 (p40 and p70), IL-17, IL-21, IL-23, IP-10, MCP-1, MCP-2, RANTES (regulated on activation, normal T cell expressed and secreted), TNF-α, and TNF-related apoptosis-inducing ligand (TRAIL). The levels of each cyto/chemokine were calculated using a standard curve. For experimental

values above the range of the standards used to calculate cyto/chemokine concentration, values were extrapolated based on the mathematical formula of the curve. Values that were out of range below the curve's lower limit were assigned a value of 0.

Serological testing. Anti-dsDNA antibodies were measured by Farr assay, RNA-associated autoantibodies by a multiplexed bead assay or ELISA, and complement factor 3 (C3) and C4 by nephelometry in the hospital laboratory.

Statistical analyses. The Mann-Whitney U test was used for comparisons of gene and cyto/chemokine expression between groups. Given multiple comparisons, a Bonferroni correction was applied to these analyses, and p values < 0.003 were considered significant. For demographic data, 2-sample Student t tests were used. For analysis of correlations between variables, a multivariate logistic regression model was built. Given the small sample size with considerable data spread, data were transformed to percentiles to maintain rank, but decrease sensitivity to extreme values.

RESULTS

Patient demographics. Table 1 shows the patient demographics for the 3 study populations examined. Age at diagnosis did not differ between SACQ and SACA or SACQ and SQCQ patients. SACQ patients were older and had longer disease duration at study start than did SACA patients. SACQ and SACA patients presented similarly at first clinic visit, with no difference in SLEDAI-2K score. Damage accrual to study start, as measured by the Systemic Lupus International Collaborating Clinics/ACR damage index, did not differ between the 3 groups; however, this was not adjusted for disease duration. Both SACQ and SQCQ patients had prolonged disease quiescence prior to study, with the duration since last flare (defined as a change in disease activity that required escalation of therapy) being on average over 10 years.

Anti-Ro and anti-La antibodies were significantly more prevalent in SACQ than in SACA patients, whereas anti-RNP antibodies were significantly more prevalent in SACA than in SACQ patients. There was no difference between SACQ and SQCQ patients in the prevalence of these autoantibodies. There were also no between-group differences in the prevalence of anti-Sm, anti-Jo1, antineutrophil cytoplasmic antibody, antiphospholipid antibodies, or lupus erythematosus cells (Table 1; data not shown). By definition, SQCQ patients had normal serum complements. There was no difference in the proportion of SACQ and SACA patients that had low complements, suggesting that both sets of patients have immune complexes.

There were no differences in SLE clinical manifestations between groups (data not shown). There was a trend toward fewer SACQ patients receiving corticosteroids (SACA 88.4%, SACQ 68.2%, p = 0.06) and significantly fewer SACQ patients receiving immunosuppressive medications over the course of their disease than SACA patients (SACA 67.4%, SACQ 31.8%, p = 0.007). The majority of patients had been treated with antimalarials over their disease course, with no difference in frequency between groups (SACA 83.7%, SACQ 81.8%, p = 0.60). At the time of sampling, 35 (81.4%) of the SACA patients were taking prednisone, with a mean and median dose of 47.9 mg and 20 mg, respectively. One SACA patient each was taking cyclophosphamide, cyclosporine, or methotrexate (2.3%), 11 were taking azathioprine (25.6%), and 12 mycophenolate (27.9%).

IFN-induced gene expression is reduced in SACQ patients.

Table 1. Patient demographics*. Values are mean ± SD unless otherwise specified.

Characteristic	SACQ, n = 22	SQCQ, n = 27	SACA, n = 43	p, SACQ vs SQCQ	p, SACQ vs SACA
Female, n (%)	19 (86.4)	26 (96.3)	37 (86.1)	0.31	1
Age at diagnosis, yrs	25.2 ± 9.9	30.8 ± 12.0	21.6 ± 9.8	0.08	0.17
Age, yrs, study start	43.7 ± 13.7	55.0 ± 12.3	28.7 ± 9.4	0.004	< 0.0001
Disease duration, study start	18.5 ± 12.5	24.2 ± 11.1	7.12 ± 7.0	0.1	0.0005
SLICC damage index, study start	n = 22, 1.05 ± 1.68	n = 27, 1.70 ± 1.91	n = 39, 0.95 ± 1.4	0.21	0.81
Adjusted mean SLEDAI, study start	3.57 ± 1.37	1.89 ± 1.34	12.85 ± 7.56	< 0.0001	< 0.0001
SLEDAI-2K at first visit	10.77 ± 10.93	6.30 ± 5.50	12.67 ± 8.70	0.09	0.45
Mean duration since last flare	13.0 ± 7.4	16.9 ± 7.8	N/A	0.07	N/A
Ethnicity, n (%)					
White	12 (54.6)	22 (81.5)	18 (41.9)	0.04, white vs others	0.33, white vs others
Black	0 (0)	4 (14.8)	12 (27.9)		
Asian	4 (18.2)	1 (3.7)	10 (23.3)		
Other	6 (27.3)	0 (0)	3 (7.0)		
ANA, n (% positive)	22 (100)	25 (92.6)	40 (100)	0.49	N/A
Anti-Sm, n (% positive)	6 (27.3)	10 (37.0)	17 (43.6)	0.47	0.21
Anti-dsDNA, Farr, n (% positive)	20 (90.9)	19 (70.4)	40 (93.0)	0.15	1
Anti-Ro, n (% positive)	18 (81.8)	16 (59.3)	18 (46.2)	0.09	0.007
Anti-La, n (% positive)	11 (50.0)	9 (33.3)	5 (12.8)	0.24	0.002
Anti-RNP, n (% positive)	8 (36.4)	7 (25.9)	29 (74.4)	0.43	0.004
C3, n (% reduced)	15 (68.2)	N/A	34 (79.1)	N/A	0.37
C4, n (% reduced)	10 (45.5)	N/A	27 (62.8)	N/A	0.20

* All durations measured in years. SACQ: serologically active clinically quiescent; SQCQ: serologically quiescent clinically quiescent; SACA: serologically active clinically active; SLICC: Systemic Lupus International Collaborating Clinics; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLEDAI-2K: SLEDAI 2000; ANA: antinuclear antibodies; C3: complement factor 3; C4: complement factor 4; N/A: not applicable.

To determine whether clinical quiescence is associated with reduced levels of IFN-induced gene expression in SACQ patients, the levels of expression of 5 IFN-induced genes were compared with those in SACA patients (Figure 1). When the genes were studied individually, trends to decreased gene expression were seen for all genes examined, but only achieved statistical significance for *LY6E* after applying the specified Bonferroni correction. Of the remaining genes, the biggest differences between groups were seen for *OAS1* and *IFIT1*, paralleling our previous results comparing unselected active and inactive patients with SLE[11]. In general, the levels of gene expression for the individual IFN genes correlated with each other and with the IFN-5 score. The SACQ IFN-5 score was also significantly lower than that of SACA patients. Notably, IFN-induced gene expression did not differ between SACQ and SQCQ patients. Indeed, means and SD between these 2 groups were nearly identical, and no notably divergent trends were observed except for, perhaps, slightly higher levels of IFN-stimulated gene 15 in SQCQ patients.

IFN-5 score is dictated by SACQ status, not autoantibody profile or disease duration. Given the proposed involvement of nuclear antigen-containing immune complexes in driving IFN-α secretion in SLE, the association between the IFN-5 score and autoantibody profile together with serum C3 levels as a surrogate marker of immune complexes was examined. As shown in Figure 2A, there was no correlation between the levels of anti-dsDNA antibodies or C3 and the IFN-5 score for either SACA or SACQ patients. However, the anti-dsDNA linear regression curves for SACA patients differed significantly from those for SACQ patients (p = 0.03), with the C3 curves demonstrating a similar trend (p = 0.06). Similarly, when SACA and SACQ patients were stratified based upon the presence or absence of Ro/La or Sm/RNP antibodies, no differences were seen between patients who were positive or negative for these autoantibodies for either patient group. Again, decreased IFN-5 gene scores were seen for SACQ as compared with SACA patients in both positive and negative autoantibody subsets, which achieved statistical significance for all but the Sm/RNP-positive patient subset. Taken together, these findings indicate that the lack of IFN signature does not arise from differences in the presence or levels of autoantibodies or immune complexes in SACQ patients.

As the disease duration was significantly longer for SACQ than SACA patients, and the IFN signature has been shown

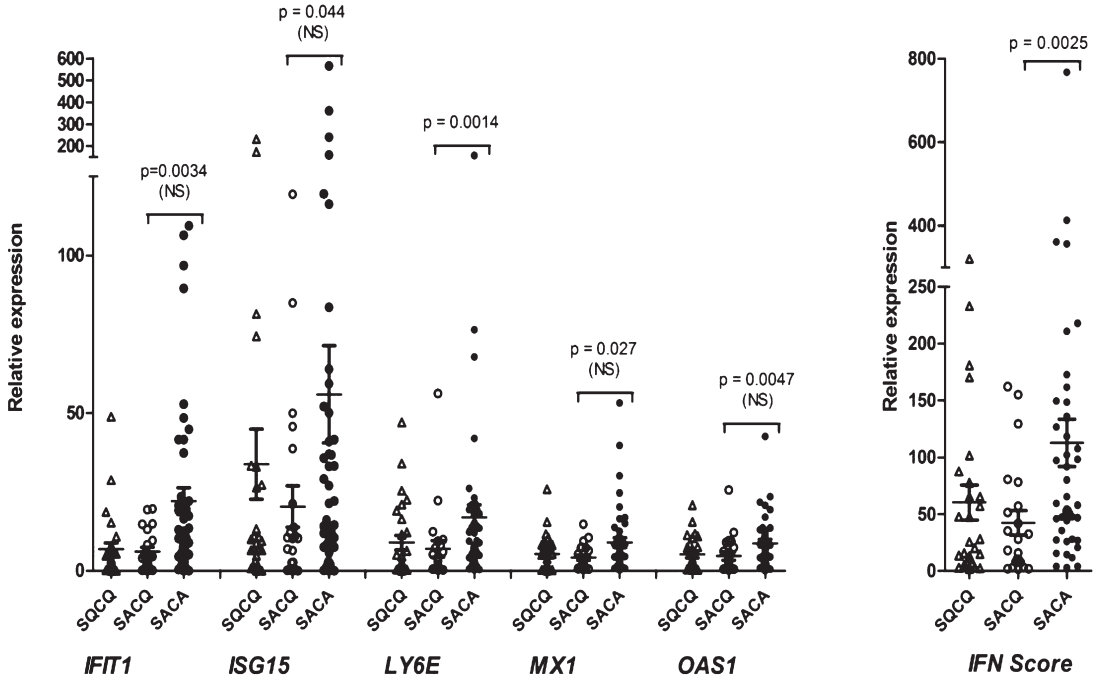

Figure 1. IFN-induced gene expression in SQCQ, SACQ, and SACA patients. Left graph: levels of expression of 5 IFN-induced genes in the indicated patient subsets. Gene expression was measured in the whole peripheral blood by qRT-PCR and normalized to *GAPDH* expression. Right graph: composite IFN score calculated as the sum of expression levels of the 5 IFN-induced genes. Each symbol represents the determination for an individual patient with horizontal lines indicating the mean for each patient subset and error bars the SEM. Statistically significant differences between groups were determined using the Mann-Whitney U test. Only statistical comparisons between SACQ and SACA patients are shown on the figure. Following Bonferroni correction for multiple comparisons, p values < 0.003 were considered to be significant. IFN: interferon; SQCQ: serologically quiescent clinically quiescent; SACQ: serologically active clinically quiescent; SACA: serologically active clinically active; qRT-PCR: quantitative real-time PCR; SEM: standard error of the mean.

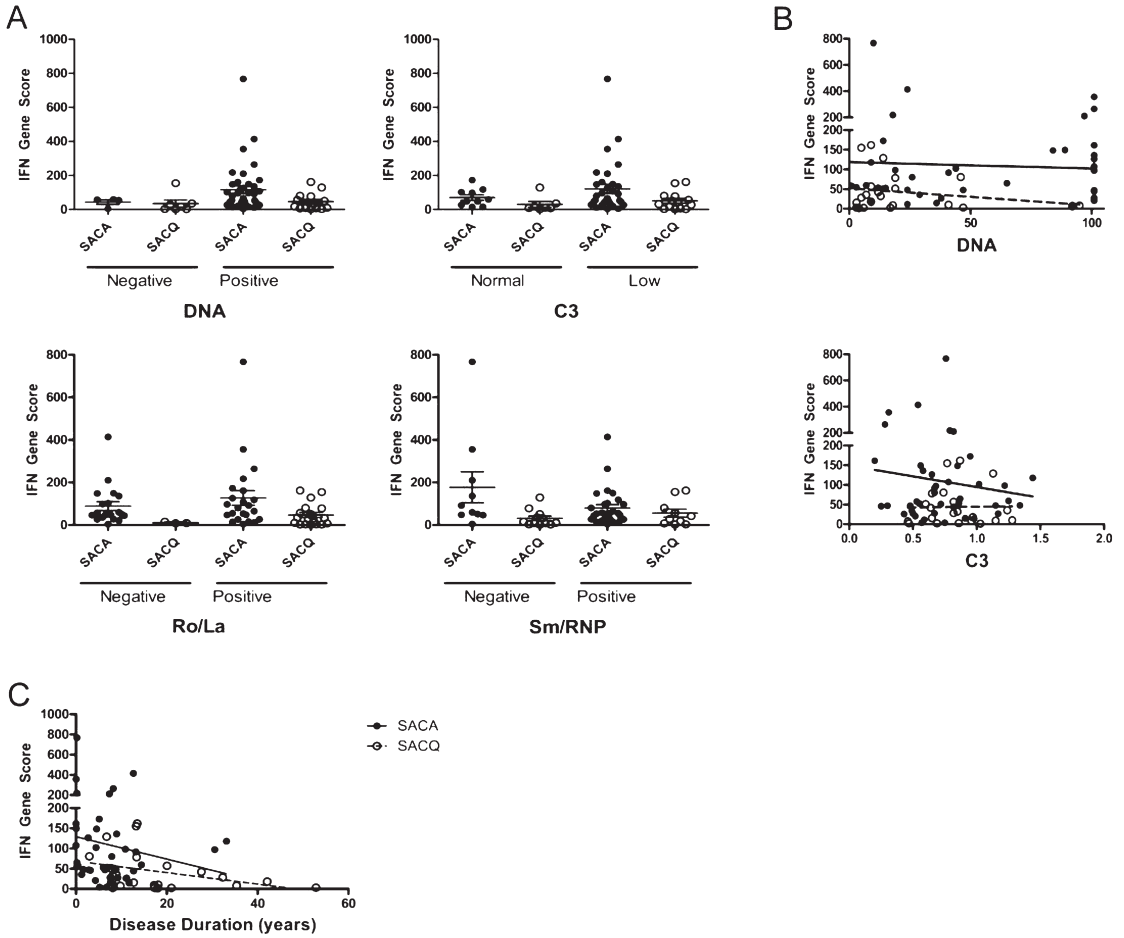

Figure 2. Correlation between composite IFN score and clinical/serologic variables in SACQ and SACA patients. (A) Association between the presence or absence of dsDNA, Ro/La or Sm/RNP antibodies, or low C3 levels, and IFN score. (B) Correlation between anti-dsDNA and C3 levels and IFN score for SACQ (open circles, dashed line) and SACA (filled circles, solid line) patients. (C) Correlation between disease duration and IFN score. Each symbol represents an independent patient. Lines of best fit for linear regression are shown for continuous variables. In scatterplots, horizontal lines indicate the mean for each patient subset and error bars the SEM. IFN: interferon; SACQ: serologically active clinically quiescent; SACA: serologically active clinically active; C3: complement factor 3; SEM: standard error of the mean.

to negatively correlate with disease duration[29], we questioned whether the reduced IFN-5 levels in SACQ patients were simply because of their longer disease duration. To address this possibility, the IFN-5 score was plotted as a function of disease duration for each group (Figure 2B). For both groups, there was a nonsignificant trend to decreased IFN-5 gene scores with disease duration; however, as shown by the linear regression lines, there was a trend to increased IFN-5 levels at all disease durations in SACA patients as compared with SACQ patients ($p = 0.2$).

To determine whether those SACQ patients with higher IFN signatures were more likely to flare, we performed a chart review of those patients with an IFN-5 score in the upper quartile over an average of 5 clinic visits and 1.5 years. All the patients remained SACQ at their most recent clinic visit. Examination of other clinical and laboratory characteristics of these patients revealed no shared features that could provide an explanation for their high levels of IFN-induced gene expression, including age, ethnicity, treatment with hydroxychloroquine, presence of infection, elevated inflammatory markers, or duration since last flare.

Proinflammatory cyto/chemokines are reduced in SACQ patients. To assess whether SACQ patients have reduced levels of proinflammatory cyto/chemokines, the plasma levels of 19 cyto/chemokines were examined. These included cytokines produced by proinflammatory T cell subsets (IL-2, IFN-γ, IL-17, IL-21), cyto/chemokines associated with production of and/or induced by IFN-α (IFN-α, GM-CSF, MCP-1, MCP-2, IP-10, RANTES, TRAIL), and other cytokines produced in response to immune complex activation of monocytes (IL-1α, IL-1β, IL-6, IL-10, IL-12 p40, IL-12 p70, IL-23, TNF-α). As shown

in Figure 3, the concentrations of 6 cyto/chemokines were significantly lower in SACQ patients as compared with SACA patients after a Bonferroni correction was applied. These were GM-CSF, IL-6, IL-10, IP-10, MCP-1, and TNF-α. There were also trends to lower concentrations of IFN-α ($p = 0.006$), IFN-γ ($p = 0.010$), IL-1α ($p = 0.023$), IL-12 p70 ($p = 0.045$), and IL-17 ($p = 0.016$); however, these did not meet the predetermined threshold for statistical significance for this dataset. Of the cyto/chemokines that significantly differed between the SACQ and SACA groups, only IL-10 levels correlated with the IFN-5 score overall ($r = 0.310$, $p = 0.002$) and in SACQ patients specifically ($r = 0.535$, $p = 0.01$). There were no differences in the levels of any of the cytokines or chemokines measured between SACQ and SQCQ patients.

There was a loose correlation between the levels of the various cyto/chemokines in SACQ patients. There was no correlation between age, disease duration, ethnicity, treatment with hydroxychloroquine, duration since last flare, and the levels of any of the cyto/chemokines. Four of SACQ patients had high levels of multiple cyto/chemokines in their plasma. There were no consistent clinical or serologic characteristics that distinguished these patients from the rest of the SACQ patients.

In a multivariable model incorporating autoantibody profile, IFN-induced genes, and cyto/chemokine levels, through stepwise logistic regression, SACQ status was most closely associated with anti-La antibody positivity, and low levels of MCP-1 and *LY6E* (Table 2).

DISCUSSION

In our study we show that, despite the presence of anti-dsDNA antibodies and/or hypocomplementemia, SACQ patients have reduced levels of IFN-induced genes and proinflammatory cyto/chemokines, similar to those observed in SQCQ patients. These findings suggest that the IFN gene signature and levels of proinflammatory cyto/chemokines parallel disease quiescence in SACQ patients better than autoantibody levels and complement, providing further support to the literature promoting measurement of these factors as biomarkers for SLE disease activity over traditional serologic biomarkers.

While it is true that the small sample sizes in both the SACQ and SQCQ groups may have limited our ability to differentiate between these 2 groups on the basis of their IFN-induced gene expression or cyto/chemokine profiles, striking consistency was noted for the majority of measures in these 2 groups. Further, for those measures that demonstrated small differences, the levels of proinflammatory factors/gene expression were actually somewhat higher in SQCQ as compared with SACQ patients. Practically speaking, a sample size large enough to explore these subtle differences would be unlikely to be achieved, even in the context of a multinational, multisite study of these very rare phenotypes.

In contrast to previous reports of unselected SLE populations, the low levels of IFN-induced gene expression in SACQ patients did not correlate with autoantibody type or levels, the absence of complement-activating immune

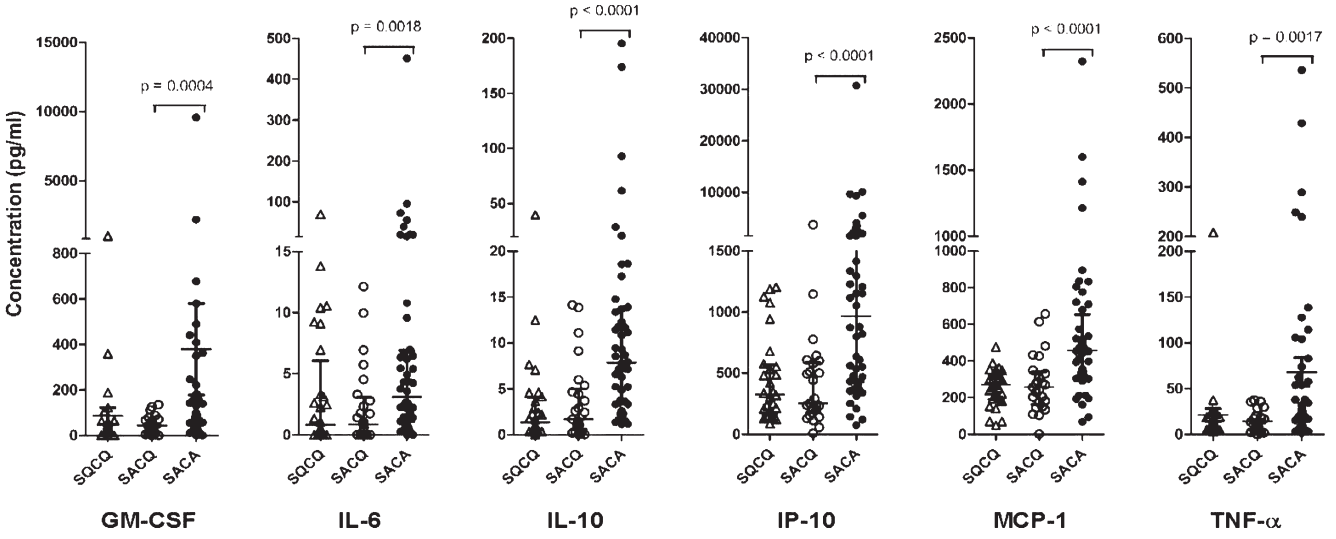

Figure 3. Plasma proinflammatory cyto/chemokine levels in SQCQ, SACQ, and SACA patients. Scatterplots are shown for the 6 cyto/chemokines of the 19 tested that demonstrated statistically significant differences between SACQ and SACA patients when subjected to a Bonferroni correction for multiple comparisons ($p < 0.003$). Cyto/chemokine levels were measured by Luminex assay. Each symbol represents the determination for an individual patient, with horizontal lines indicating the mean for each patient subset and error bars the SEM. Statistically significant differences between groups were determined using the Mann-Whitney U test. Only statistical comparisons between SACQ and SACA patients are shown. SQCQ: serologically quiescent clinically quiescent; SACQ: serologically active clinically quiescent; SACA: serologically active clinically active; SEM: standard error of the mean.

Table 2. Associations with SACQ status by logistic regression analysis.

Variable	OR (95% CI)	p
Anti-La positivity	168.1 (4.85 to > 999)	0.005
MCP-1 high	0.87 (0.80–0.96)	0.003
Ly6e high	0.90 (0.85–0.97)	0.004

SACQ: serologically active clinically quiescent; MCP-1: monocyte chemoattractant protein 1.

complexes, or disease duration. These findings argue that the reduced IFN signature in SACQ patients is not a result of differences in these serologic and clinical features between SACQ and SACA patients, but instead may provide insight into the immune mechanism(s) leading to SACQ status itself. Interestingly, previous work suggests that the levels of IFN-induced gene expression may be genetically determined[13,14]. Further, we and others have shown that although the IFN signature correlates with autoantibody positivity and disease activity in cross-sectional studies, it does not fluctuate greatly between visits over time, despite changes in disease activity[11,12]. This has led to the concept that the association between disease activity and high levels of IFN-induced gene expression observed in cross-sectional studies results from generally increased disease severity with more frequent flares in patients who have genetic polymorphisms promoting higher levels of IFN-induced gene expression[13,30]. Thus, it is possible that the patients with SLE who ultimately develop SACQ (or SQCQ) may have had lower levels of IFN throughout their disease course, resulting in an increased likelihood of establishing disease quiescence. The observation that the IFN signature is lower for SACQ (and SQCQ; data not shown) patients as compared with SACA patients at all disease durations provides support for this concept. Conversely, high levels of IFN alone are insufficient to promote disease activity and must act in the appropriate autoimmune context, as evidenced by observation of high levels of IFN activity in the first-degree relatives of patients with SLE who are healthy with no evidence of autoimmune disease[13].

Consistent with the low levels of IFN-induced gene expression in SACQ patients, the levels of IFN-α together with other IFN-induced cytokines, IP-10 and MCP-1, were reduced in this patient population. While IFN-α has not been shown to directly induce expression of GM-CSF, IL-10, or TNF-α, it is possible that the reduced levels of these cytokines are also related to the lack of IFN-α in SACQ patients because anti-IFN-α antibody-treated patients with SLE had reduced levels of GM-CSF–, IL-10–, and TNF-α–induced gene expression following treatment[31]. A number of the other cyto/chemokines that were significantly reduced (IL-6) or showed a trend to reduction (IFN-γ, IL-17, IL-1α, IL-12) have been shown to be induced by uptake of immune complexes or apoptotic debris by monocytes/dendritic cells[28,32]. It is currently unclear whether the levels of these cytokines could also be indirectly affected by the lack of IFN-α in SACQ patients, or whether the reduced levels of these cyto/chemokines reflects a more general process impairing proinflammatory cyto/chemokine production in response to immune complexes in SACQ patients.

Although the majority of SACQ patients had low levels of proinflammatory factors, occasional patients had levels of 1 or more of these molecules that were at or above the mean for SACA patients. In general, the elevations in these molecules did not correlate with each other, and did not predict those patients who were more likely to flare in the subsequent 18 months. Whether these patients consist of the ~60% SACQ patients that will ultimately flare is currently unknown because none of the SACQ patients who participated in our study have flared yet. However, a longitudinal study of this cohort could provide insight into the immune events that forecast flare in this subset.

Because current concepts of disease pathogenesis place nuclear antigen-containing immune complexes centrally in the production of IFN-α and other proinflammatory factors by pDC, the lack of these factors in SACQ patients raises questions regarding the importance and/or regulation of this process. Although the RNA-associated autoantibody profile differed between SACQ and SACA patients, it is unlikely that this resulted in the differences in proinflammatory factor levels between these 2 patient populations because both anti-Ro/La antibody-containing and anti-RNP antibody-containing immune complexes are well documented to stimulate pDC[18,33,34]. It also appears unlikely that the reduced levels of proinflammatory cytokines arise from a lack of nuclear antigens bound to these antibodies in SACQ patients since many of the SACQ patients had low levels of C3; and even those patients with lowest levels of C3 had reduced cyto/chemokine levels as compared with SACA patients.

Nevertheless, we cannot rule out the possibility that the character of the nuclear antigens that are present in SACQ patients differs from that for SACA patients. Previous studies suggest that there are several potential sources of nuclear antigens in SLE, including apoptotic debris, necrotic debris, and neutrophil extracellular traps (NET), that differ in their capacity to induce proinflammatory cytokines. For example, patients with active SLE have higher levels of NET, which have been shown to directly activate pDC to secrete proinflammatory cytokines and which could augment cyto/chemokine production in SACA patients[35].

Alternatively, SACQ patients may have developed immunologic mechanisms that act to impair production of proinflammatory factors despite the presence of relevant immune complexes. In this regard, C1q and C-reactive protein have been shown to inhibit the pDC response to immune complexes[36,37]. It is unlikely, however, that high levels of these factors lead to inhibition of proinflammatory factor production in SACQ patients because these patients have evidence of complement activation and generally

normal levels of CRP. Two more likely possibilities are that SACQ patients have high levels of nucleases that act to rapidly degrade nuclear antigen-containing immune complexes or that they have anticytokine antibodies. In connection with the latter possibility, previous work has shown that patients with SLE can produce a variety of anti-cytokine antibodies, with a study finding a higher prevalence of anti-IFN-α antibodies in a small cohort of SACQ patients (4/11, 36%) as compared with the general SLE population (9%)[38]. Finally, it is possible that there are cellular mechanisms in SACQ patients, such as inhibition by monocytes[39], that lead to impaired production of proinflammatory cytokines by their pDC. In ongoing experiments, we are seeking to discriminate between these various possibilities.

Our findings suggest that it may not be necessary to restore tolerance and prevent autoantibody production to treat SLE, provided that the production of downstream proinflammatory factors can be blocked. If this concept is valid, then therapies that influence the capacity of innate immune populations to produce these factors, such as those that block the Toll-like receptor-signaling pathway or the Type I IFN that augment these processes, should be effective. As trials examining some of these therapies are already in progress, it will be of great interest to observe whether their results provide support for this concept.

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