Published in final edited form as:

J Autoimmun. 2017 March ; 78: 70–78. doi:10.1016/j.jaut.2016.12.005.

Pathways of Impending Disease Flare in African-American Systemic Lupus Erythematosus Patients

Melissa E. Munroe^a, Evan S. Vista^{a,b}, Joan T. Merrill^a, Joel M. Guthridge^a, Virginia C. Roberts^a, and Judith A. James^{a,c}

^aArthritis and Clinical Immunology, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104 USA

^bSt. Luke's Medical Center, Taguig, PH 1112

^cDepartments of Medicine and Pathology, Oklahoma Clinical and Translational Science Institute, University of Oklahoma Health Sciences Center, 940 Stanton L. Young Boulevard, BMSB 451, Oklahoma City, OK 73104 USA

Abstract

Immune dysregulation in systemic lupus erythematosus (SLE) contributes to increased disease activity. African American (AA) SLE patients have an increased prevalence of complications from disease flares and end-organ damage that leads to increased morbidity and early mortality. We previously reported alterations in inflammatory and regulatory immune mediator levels prior to disease flare in European American (EA) SLE patients. In the current study, we assessed baseline and follow-up plasma levels of 52 soluble mediators, including innate, adaptive, chemokine, and TNF superfamily members, in AA SLE patients who developed SELENA-SLEDAI defined flare 6 or 12 weeks after baseline assessment. These patients were compared to themselves during a comparable, clinically stable period (SNF, n=18), or to demographically matched SLE patients without impending disease flare (NF, n=13 per group). We observed significant ($q < 0.05$) alterations in 34 soluble mediators at baseline, with increased levels of both innate (IL-1 α and type I interferons [IFN]) and adaptive cytokines (Th1-, Th2-, and Th17-type), as well as IFN-associated chemokines and soluble TNF superfamily members weeks before clinical disease flare. In contrast, stable SLE patients exhibited increased levels of the regulatory mediators IL-10 ($q = 0.0045$) and TGF- β ($q = 0.0004$). Because heterogeneous immune pathways were altered prior to clinical disease flare, we developed a soluble mediator score that encapsulates all mediators tested. This score is the sum of all log transformed, standardized soluble mediator levels assessed at baseline (pre-flare), weighted by their Spearman correlation coefficients for association with the

Correspondence to: Judith A. James, MD, PHD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, Phone: 405-271-4987, Fax: 405-271-7063, judith-james@omrf.org.

Author Contributions

JMG, VCR, JTM, and JAJ assembled the SLE Influenza Vaccination Cohort and assembled part of the dataset. ESV, JTM, and JAJ collected clinical patient data. MEM and JAJ designed and carried out the soluble mediator experiments. MEM and JTM carried out the statistical analysis. All authors were involved in the writing, revision, and approval of the manuscript. All authors read and approved the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

SELENA-SLEDAI score at time of concurrent flare. While baseline SELENA-SLEDAI scores were similar between flare vs. NF ($p=0.7214$) and SNF ($p=0.5387$), the SMS was significantly higher in pre-flare SLE patients (Flare vs NF or SNF, $p<0.0001$). By capturing alterations in the balance between inflammatory and regulatory mediators associated with SLE pathogenesis, the soluble mediator score approximates the immune status of SLE patients and provides a robust, predictive gauge of impending disease flare.

Graphical Abstract

Keywords

Systemic Lupus Erythematosus; Acute Symptom Flare; Cytokines; Chemokines; TNF Superfamily; African Americans

1. Introduction

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by chronic immune dysregulation [1]. Disease activity in SLE often waxes and wanes, with flare defined by validated clinical instruments, including the Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SELENA-SLEDAI [2]). Despite improved treatment regimens and disease outcomes [3], SLE patients may experience an average of 1.8 disease flares annually [4] that require the use of rapidly acting, potentially toxic agents such as corticosteroids.

The ability to predict impending disease flare would allow for earlier treatment to mitigate or prevent flare-associated organ damage that contributes to increased morbidity and early mortality in SLE patients [5], while also potentially improving quality of life for SLE patients [6]. This would be particularly useful in African American (AA) SLE patients, who frequently experience a more aggressive disease course. AA SLE patients face an increased risk of developing irreversible organ system involvement, including permanent CNS, pulmonary, and cardiovascular damage [7–10], lupus nephritis and end-stage renal disease

[11], and a threefold increase in SLE-related mortality compared to European American (EA) patients [12].

Multiple inflammatory and regulatory mediators are known to be involved in SLE pathogenesis and disease flare, including innate [13] and adaptive cytokines [14], chemokines [15], and altered regulation of soluble receptors [16, 17] expressed by activated immune cells. As varied immunological pathways influence disease activity across SLE patient populations, a comprehensive immune mediator panel may be required to monitor immune function and flare risk. Such is the case in rheumatoid arthritis (RA), where a panel of 12 RA-associated soluble mediators has been identified that allows for rapid, reliable, and objective assessment of joint damage risk and response to therapy [18]. In EA SLE patients, we have previously shown that multiple immune mediators change significantly up to 12 weeks prior to disease flare, and that a soluble mediator score (SMS) integrating plasma levels of 52 cytokines and chemokines accurately identifies impending disease flare in EA SLE patients [19]. In the current study, we explored inflammatory and regulatory soluble mediators potentially dysregulated before clinical symptoms of SLE disease flare and tested the ability of the SMS to differentiate impending disease flare in AA SLE patients.

2. Materials and Methods

2.1. Study population

Experiments were performed in accordance with the Helsinki Declaration and approved by the Institutional Review Boards of the Oklahoma Medical Research Foundation and the University of Oklahoma Health Sciences Center. Study participants were enrolled in the SLE Influenza Vaccination Cohort [20] after written informed consent. AA SLE patients (meeting > 4 ACR classification criteria [20]) with disease flare 6 or 12 weeks post-baseline evaluation (Flare, n=13) were matched by age (± 5 years), race, gender, and time of disease assessment to 13 patients with stable disease (nonflare, NF) and 13 healthy controls (HC). Samples from 18 AA pre-flare SLE patients (Flare) were compared to samples drawn from the same individuals in a different year with no associated SELENA-SLEDAI flare 6 or 12 weeks post-baseline assessment (self non-flare, SNF), as well as 18 healthy controls matched by age (± 5 years), race, and gender.

2.2. Clinical data and sample collection

Demographic and clinical information were collected as previously described [20], including humoral response to influenza vaccination, medication usage, clinical laboratory values, disease activity, and SELENA-SLEDAI [2] defined flare (Table 1). SELENA-SLEDAI disease activity was assessed at baseline (pre-vaccination) and again at 6 and 12 weeks post-vaccination (follow-up). The presence of system involvement was evaluated by the administration of the SELENA-SLEDAI disease activity instrument, including the presence of disease manifestations involving the central nervous system (CNS; seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, or lupus headache), vasculitis, arthritis, myositis, nephritis (urinary casts, hematuria, proteinuria, or pyuria), mucocutaneous damage (rash, alopecia, or mucosal ulcers), serositis (pleuritis or pericarditis), or hematologic manifestations (low complement, increased DNA binding,

fever, thrombocytopenia, or leukopenia) [2]. Blood samples were procured from each participant at baseline (pre-vaccination), and at 6 and 12 weeks of follow-up (post-vaccination). Plasma samples were stored at -20°C until time of assay. Assays were performed on freshly thawed samples.

2.3. Measurement of soluble mediators and autoantibody specificities

Plasma soluble mediators (n=52, Supplemental Tables 1–2) were examined, including cytokines, chemokines, and soluble receptors, using validated multiplex bead-based assay or ELISAs (BLyS and APRIL) [21]. Data were analyzed on the Bio-Rad BioPlex 200[®] array system (Bio-Rad Technologies, Hercules, CA), with a lower boundary of 100 beads per sample/analyte. Median fluorescence intensity for each analyte was interpolated from 5-parameter logistic nonlinear regression standard curves. Analytes below the detection limit were assigned a value of 0.001 pg/mL. Well-specific validity was assessed by AssayCheX[™] QC microspheres (Radix BioSolutions, Georgetown, TX, USA) to evaluate non-specific binding. A known control serum was included on each plate (Cellgro human AB serum, Cat#2931949, L/N#M1016). Mean inter-assay coefficient of variance (CV) of multiplexed bead-based assays for cytokine detection has previously been shown to be 10–14% [22, 23], and a similar average CV (10.5%) across the analytes in this assay was obtained using healthy control serum. Intra-assay precision of duplicate wells averaged <10% CV in each 25-plex assay.

Plasma samples were screened for autoantibody specificities using the BioPlex 2200 multiplex system (Bio-Rad Technologies, Hercules, CA). The BioPlex 2200 ANA kit uses fluorescently dyed magnetic beads for simultaneous detection of 11 autoantibody specificity levels, including reactivity to dsDNA, chromatin, ribosomal P, Ro/SSA, La/SSB, Sm, the Sm/RNP complex, RNP, Scl-70, centromere B, and Jo-1 [24]. SLE-associated autoantibody specificities to dsDNA, chromatin, Ro/SSA, La/SSB, Sm, Sm/RNP complex, and RNP were used for analysis in the current study. Anti-dsDNA (IU/mL) has a previously determined positive cutoff of 10 IU/mL; an Antibody Index (AI) value (range 0–8) is reported by the manufacturer to reflect the fluorescence intensity of each of the other autoantibody specificities with a positive cutoff of AI=1.0. The AI scale is standardized relative to calibrators and control samples provided by the manufacturer.

2.4. Statistical Analyses

Baseline SELENA-SLEDAI scores and plasma soluble mediator concentrations were compared between AA SLE patients with imminent disease flare and matched NF SLE patients or SNF periods by Wilcoxon's matched-pairs test. Plasma mediator concentrations at baseline and follow-up were compared between pre-flare SLE patients, matched NF or SNF samples, and matched HC samples by Friedman test with Dunn's multiple comparison. Baseline plasma mediator concentrations were correlated with SELENA-SLEDAI scores at time of flare (follow-up) in Flare/NF and Flare/SNF samples by Spearman's rank correlation. Except where noted, analyses were performed using GraphPad Prism 6.02 (GraphPad Software, San Diego, CA).

A soluble mediator score (SMS) calculation was developed to compare the overall level of inflammation at baseline in pre-flare vs. non-flare periods in relationship to disease activity at follow-up (flare). The SMS calculation for pre-flare vs. unique, demographically matched non-flare SLE patients (NF), as well as for pre-flare vs. non-flare periods within the same patient (SNF), followed an approach previously used for rheumatoid arthritis [25] and EA SLE patients [19] to summarize the dysregulation of all 52 plasma mediators assessed at baseline (pre-flare or non-flare time point, Supplemental Tables 1–2, left column), weighted by their correlation to SELENA-SLEDAI disease activity at follow-up (Supplemental Tables 1–2, center panel). For each comparison group (flare vs NF or flare vs SNF), the SMS was calculated as follows: 1. The concentrations of all 52 baseline plasma mediators, plus IL-1RA:IL-1 β ratio [26], were log-transformed for each SLE patient. 2. Each log-transformed soluble mediator level (plus IL-1RA:IL-1 β ratio) for each SLE patient was standardized: (observed value)-(mean value of all SLE patients assessed [Flare and NF or Flare and SNF])/(standard deviation of all SLE patients assessed [Flare and NF or Flare and SNF]). 3. Spearman coefficients were generated from a linear regression model testing associations between the SELENA-SLEDAI disease activity score at follow-up in each SLE patient and each soluble mediator at baseline (Spearman r, Supplemental Tables 1–2, center panel); 4. The transformed and standardized soluble mediator levels at baseline were weighted (multiplied) by their respective Spearman coefficients (Spearman r, Supplemental Tables 1–2, center panel). Soluble mediators that best distinguished pre-flare from NF or SNF patients most significantly contributed to the SMS (Supplementary Tables 1–2 (right panel). 5. For each patient, the log transformed, standardized and weighted values for each of the 52 soluble mediators, plus IL-1RA:IL-1 β ratio (Supplemental Tables 1–2, left column), were summed to calculate a total SMS.

The SMS was compared between AA SLE patients with imminent disease flare and matched NF SLE patients or SNF periods by Wilcoxon's matched-pairs test. Odds ratios (OR) were determined for the ability of each soluble mediator to positively or negatively contribute to the SMS, as well as the likelihood of a pre-flare or non-flare SLE patient to have a positive or negative SMS, respectively; significance for each OR was determined by Fisher's exact test. P-values were adjusted for multiple comparisons using the False Discovery Rate via the Benjamini-Hochberg procedure (using R version 2.15.3).

3. Results

3.1 Increased inflammatory and decreased regulatory mediators prior to impending disease flare

AA SLE patients have increased disease severity partnered with enhanced autoantibody production [7, 8, 27, 28]. Informed by our previous study wherein immune alterations occurred in EA SLE patients with imminent disease flare [19], we hypothesized that AA SLE patients would also exhibit increased immune dysregulation prior to SELENA-SLEDAI defined disease flare. Soluble mediators, including innate and adaptive cytokines, chemokines, and soluble TNF superfamily members, were assessed in plasma samples from AA SLE patients who experienced disease flare 6 or 12 weeks after baseline assessment compared to demographically-matched non-flare (NF) patients (n=13 Flare vs. 13 NF) or to

the same patients during an equivalent period of stable disease activity (n=18 Flare vs. 18 self non-flare; SNF). Whether comparing Flare vs. matched NF patients (Fig. 1 and Supplemental Table 1, left panel) or Flare vs SNF periods within the same patient (Fig. 2 and Supplemental Table 2, left panel), baseline/pre-flare levels of 34 of 52 soluble mediators assessed were significantly higher in the pre-flare group. Increased plasma levels of immune mediators in the Flare group included innate pathways, such as IL-1 α and type I interferon ([IFN]- β), as well as adaptive pathways, such as Th1-associated mediators (IL-12(p70) and soluble IL-2R α) and Th17-associated mediators (IL-6, IL-17A, and IL-21) (Fig. 1A–C and Fig. 2A–C). Coinciding with increased levels of pro-inflammatory mediators, the Flare group exhibited significantly lower levels of the regulatory mediator TGF- β (Figs. 1D and 2D). In addition, patients with impending flare had significantly higher levels of IFN-associated chemokines (MCP-1, MCP-3, MIG, IP-10, and MIP-1 β , Figs. 1E and 2E) and soluble tumor necrosis factor (TNF) superfamily members, including CD40L and TRAIL (Figs. 1F and 2F). Of note, patients with impending flare also had increased levels of the cytokine stem cell factor (SCF; Figs. 1G and 2G). Neither BLYS nor APRIL levels were altered prior to impending disease flare, whether comparing Flare vs. matched NF SLE patients (Supplemental Table 1, left column) or Flare vs SNF periods within the same SLE patient (Supplemental Table 2, left column). Further, BLYS and APRIL levels remained consistent through the study period (Supplemental Table 3). These data suggest that immune dysregulation precedes clinical disease flare in AA SLE patients. Further, soluble mediator levels did not change significantly from baseline to follow-up (Supplemental Figs. 1–2), suggesting that this immune dysregulation persists through the time of clinical flare.

3.2 Pre-flare levels of mediators correlate with disease activity at time of subsequent clinical flare

We next evaluated whether mediators that were significantly altered at baseline in pre-flare vs. non-flare AA SLE patients correlated with follow-up SELENA-SLEDAI disease activity at the time of concurrent flare (Table 2, left panel, **and** Supplemental Tables 1–2, center panel). Baseline, pre-flare levels of several immune mediators correlated with clinical disease activity at the time of concurrent flare in AA SLE patients, even after adjusting for multiple comparisons. SELENA-SLEDAI scores at follow-up (flare), but not at baseline (pre-flare, Supplemental Table 4), correlated with increased baseline (pre-flare) levels of innate (IL-1 α) and adaptive (Th1 and Th17) mediators, as well as chemokines (IL-8/CXCL8, IFN-associated chemokines MCP-3/CCL7, MIG/CXCL9, IP-10/CXCL10, and the adhesion molecule, ICAM-1), TNF superfamily members (sCD40L and TRAIL), and the pro-inflammatory mediator SCF. Conversely, SELENA-SLEDAI scores at the time of concurrent flare (follow-up) were associated with decreased baseline levels of the regulatory mediator TGF- β (Table 2, left panel, **and** Supplemental Tables 1–2, center panel). These findings suggest that there may be a direct connection between immune dysregulation and impending clinical disease flare in AA SLE patients.

3.3 A Soluble Mediator Scores distinguish impending disease flare in AA SLE patients

Clinical disease activity at baseline, quantified as SELENA-SLEDAI scores, did not significantly differ between Flare and NF SLE patients (Fig. 3A), nor between Flare and SNF periods within the same AA SLE patients (Fig. 3B). Likewise, neither the presence of

lupus-associated autoantibody specificities, medication usage, nor ESR levels were significantly different between Flare and NF patients or SNF periods at baseline (Table 1). Only at follow-up did the SELENA-SLEDAI significantly distinguish between Flare (7.3 ± 2.9) and NF (2.9 ± 3.0) SLE patients ($p=0.0002$) or between Flare (8.4 ± 6.2) and SNF (4.1 ± 3.7) periods within the same SLE patient ($p=0.0020$; Table 1), including significant increases in SELENA-SLEDAI mucocutaneous symptoms in Flare vs NF patients ($p=0.0414$; Table 1).

Given the significant alterations in multiple immune pathways prior to clinical disease flare in EA SLE patients, we previously developed soluble mediator scores (SMS) to summarize the immune dysregulation in individual patients, comparing pre-flare vs. unique, demographically matched non-flare SLE patients, as well as pre-flare vs. non-flare periods of disease activity in the *same* SLE patient [19]. Rather than requiring positive cutoffs for each soluble mediator, the SMS is calculated using log-transformed, normalized levels of each baseline (pre-flare) soluble mediator (and IL-1 β :IL-1RA ratio [26]) weighted based on their correlations to disease activity at follow-up (time of clinical flare). The sum of the weighted, log-transformed, normalized levels for each analyte produces the global SMS (please see Materials and Methods for details). We applied the same SMS process developed for EA SLE patients [19] to the baseline/pre-flare data for the AA SLE patients in the current study (Supplemental Fig. 3A–B). The SMS was significantly higher in AA Flare compared to NF SLE patients (median SMS 7.41 [Flare] vs. -8.46 [NF]; OR, 729 [95%CI, 13.4–13518]; $p<0.0001$, Fig. 3C and Table 3), exhibiting high sensitivity (1.0) and specificity (1.0) in distinguishing AA SLE patients with imminent flare from those with stable disease (AUC = 1, $p<0.0001$, Supplemental Fig. 3C). Similarly, comparing pre-flare and SNF periods within the same AA SLE patient, the SMS was able to distinguish Flare vs. SNF with high sensitivity (1.0) and specificity (0.8889, AUC = 0.9907, $p<0.0001$, Supplemental Fig. 3D). All 18 patients exhibited higher SMS scores prior to disease flare, with an average SMS increase of 8.0 ± 3.0 (SD) above that of the SNF period (median SMS 4.09 [Flare] vs. -4.01 [SNF]; OR, 164 [7.8–3425]; $p<0.0001$, Fig. 3D and Table 3). Significant differences in pre-flare levels of a number of innate, adaptive, chemokine, and TNF superfamily mediators, coupled with their significant correlation to future disease activity at time of concurrent flare, resulted in significant contributions of these varied mediators to the SMS (Table 2, right panel, **and** Supplemental Tables 1–2, right panel). In addition, when soluble mediator levels from AA SLE patients (current study) were merged with those from EA SLE patients [19], the SMS distinguished SLE patients with imminent disease flare across ethnicities (Supplemental Fig. 4). Similar to EA SLE patients [19], pre-flare AA SLE patients were found to have increased levels of Th17-type adaptive mediators (e.g. IL-6, IL-17A, and IL-21), IFN-associated chemokines (e.g. MCP-1/CCL2, MCP-3/CCL7, and IP-10/CXCL10), and TNF superfamily mediators (e.g. FasL, CD40L, and TNFR2) that significantly contributed to the SMS (Supplemental Tables 1–2 and [19]). Unlike pre-flare EA SLE patients, who had significant alterations in innate IL-1 family members that contributed to the EA SMS [19], AA patients had increased levels of innate type I IFN levels that contributed to the AA SMS (Supplemental Tables 1–2). These data support the ability of the SMS to distinguish AA SLE patients with imminent disease flare by integrating the heterogeneous nature of pre-flare immune dysregulation.

4. Discussion

A pro-active approach is necessary to manage immune dysregulation in SLE. Validated disease activity clinical instruments, such as the SELENA-SLEDAI, assess and weigh changes in signs and symptoms within each organ system and are reliable measures of clinical disease activity [1]. However, clinical disease flares are only detected after uncontrolled inflammation contributes to the accrual of tissue and permanent end-organ damage that can result in increased morbidity and early mortality for AA SLE patients. No serologic prognostic tool currently exists to identify SLE patients at risk of imminent disease flare in order to pre-emptively intervene. This study expands our earlier findings in EA SLE patients by demonstrating that AA SLE patients also exhibit a pattern of increased inflammation prior to clinical flare and display an enhanced regulatory state during a period of stable disease activity. Furthermore, a SMS informed by altered pre-flare immune mediators has high sensitivity and specificity for differentiating AA SLE patients with impending flare.

Multiple mediators representing varied immune pathways were significantly altered in pre-flare SLE patients and correlated with future disease activity at time of impending flare. That altered pre-flare mediator levels assessed at baseline did not correlate with baseline disease activity, where SELENA-SLEDAI scores were similar between pre-flare and non-flare periods of disease activity, supports our hypothesis that additional, uncontrolled, immune dysregulation occurs prior to clinical disease flare in SLE patients. A number of mediators that distinguished pre-flare AA SLE patients centered on pathways associated with innate and adaptive immunity. Alterations in type I IFN (IFN- β), Th1-type mediators associated with type II IFN (IFN- γ), and IFN-associated chemokines were unsurprising, given the well described IFN signature present in SLE patients [29]. Of note, Type I IFN mediators were more likely to be altered and contribute to the SMS in AA SLE patients, while innate mediators from the IL-1 family were more likely to be altered in EA [19] patients. In contrast, IFN-associated chemokines, including IP-10 and MCP-3, were altered and contributed to the SMS in both AA and EA [19] SLE patients. In addition, SCF levels were consistently elevated prior to disease flare and contributed to the SMS in both AA and EA [19] SLE patients. In dendritic cells, SCF signaling has been shown to promote the production of IL-6 and facilitate Th17 development, with increased secretion of IL-17A and IL-21 [30–32], all of which are elevated in pre-flare AA and EA [19] SLE patients. In contrast to the previous suggestion that Th17 activation is a poor biomarker for disease activity [33], our results in this report, as well as our previous study in EA SLE patients [19], support a role for Th17-mediated responses in SLE-related inflammation. Likewise, the elevation in IL-12 levels prior to disease flares may promote a Th1 response, and while we did not observe a significant increase in IFN- γ levels, multiple IFN- γ -induced chemokines, including IP-10, MIG, and MIP-1 β , were significantly elevated in pre-flare AA SLE patients.

In addition, a number of soluble TNF superfamily members were elevated in pre-flare AA and EA [19] SLE patients, potentially cleaved from their membranous forms in response to inflammation [34]. Notably absent were the TNF superfamily members, BLYS and APRIL; neither mediator differentiated pre-flare from non-flare periods in the current study of AA

SLE patients, nor in our previous evaluation of EA SLE patients [19]. Further, BLYS and APRIL levels remained consistent through the time of concurrent clinical flare, suggesting that they were not the primary drivers of disease flare in the SLE patients assessed in our cohort. Clinical trials of belumimab (anti-BLYS [35, 36]) and atacicept (anti-BLYS/APRIL [37]), while promising for some SLE patients in reducing number of SLE disease flares, demonstrated mixed results for their impact on SLE disease pathogenesis. This variability suggests that BLYS and APRIL may drive altered disease activity in some patients but not others [38]. This may also be true for other immune pathways.

Within the current study, we observed heterogeneity in the number and type of immune pathways altered in individual pre-flare SLE patients. This may explain, in part, the variability among previous reports of inflammatory mediators in SLE patients with active disease [39–41], as well as the inconsistent correlations with disease activity and limited clinical utility of proposed serologic markers of disease activity, alone or in combination, including anti-dsDNA, complement, complement split products, and inflammatory markers (ESR and CRP) [42–44]. Despite the heterogeneity in immune pathway involvement, each patient demonstrated elevations in inflammatory mediators from at least one pathway, with a concurrent relative decrease in regulatory mediators, prior to clinical disease flare.

Therefore, the SMS harnesses a wide breadth of immune mediator information to monitor the overall immune status and differentiate pre-flare from non-flare periods in EA [19] and AA (current study) SLE patients. This is an essential feature, as focusing on a single pathway (e.g. Type I IFN), even at the molecular level, will decrease the number of patients that can be captured in a pre-flare state [45]. By providing a broad survey of immune pathway activation, the score shows remarkable consistency despite immunologic and clinical heterogeneity among patients, similar to a soluble mediator-informed, algorithm-based laboratory test that has been validated in rheumatoid arthritis [46]. This is in contrast to traditional biomarkers incorporated in the SELENA-SLEDAI, which are not necessarily the earliest, nor sufficient biologic signals of worsening disease. Therefore, the SMS may extend the definition of serologically active disease beyond the capabilities of traditional biomarkers.

The ability to detect changes in immune status before disease flares become clinically apparent would allow for improved disease surveillance and treatment, which could improve patient outcomes and reduce the pathogenic and socioeconomic burdens of SLE [6]. An advantage of calculating a patient's SMS is that it does not require cut-offs for each soluble mediator to establish positivity, and does not require *a priori* knowledge of the inflammatory pathways that contribute to flare in a particular patient. Data from our current study, combined with our previous report in EA SLE patients [19], suggest the refinement of the SMS calculation to a single, clinically actionable, algorithm would be applicable across multiple ethnicities. Validating and refining the SMS in prospective, multiethnic studies would establish a valuable prognostic tool in SLE clinical trials and disease management. Depending on the comprehensive clinical picture of an individual patient, early detection of risk for SLE flare could prompt closer monitoring, preventative treatments, or inclusion in clinical trials for targeted biologics relevant to pathways altered within the mediator score.

5. Conclusions

Data from our current study demonstrate pro-inflammatory innate, adaptive, and TNF family mediators are elevated in pre-flare lupus patients, while regulatory mediators are elevated in AA SLE patients with stable disease. Alterations in the balance between inflammatory and regulatory mediators may help identify AA patients at risk of disease flare and help decipher SLE pathogenic mechanisms via an immune mediator informed soluble mediator score.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank all of the study participants for their time and commitment to the study. We would like to thank the referring physicians and Amy Dedeke, MD for their assistance. We would also like to thank Jourdan Anderson and Wade DeJager for clinical or technical assistance. Finally, we would like to thank Rebecka Bourn, PhD and Miles Smith, PhD for scientific editing.

Grant support

This study was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the National Institute of Allergy and Infectious Diseases with co-funding by the Office of Research on Women's Health, and the National Institute of General Medical Sciences of the National Institutes of Health under award numbers P30AR053483, U19AI082714, U01AI101934, P30GM103510, U54GM104938, and HHSN266200500026C. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Lam GK, Petri M. Assessment of systemic lupus erythematosus. *Clin Exp Rheumatol*. 2005; 23:S120–32. [PubMed: 16273796]
2. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al. Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med*. 2005; 353:2550–8. [PubMed: 16354891]
3. Urowitz MB, Gladman DD, Tom BD, Ibanez D, Farewell VT. Changing patterns in mortality and disease outcomes for patients with systemic lupus erythematosus. *J Rheumatol*. 2008; 35:2152–8. [PubMed: 18793004]
4. Petri M, Singh S, Tesfayone H, Malik A. Prevalence of flare and influence of demographic and serologic factors on flare risk in systemic lupus erythematosus: a prospective study. *J Rheumatol*. 2009; 36:2476–80. [PubMed: 19833757]
5. Rahman P, Gladman DD, Urowitz MB, Hallett D, Tam LS. Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus*. 2001; 10:93–6. [PubMed: 11237132]
6. Lau CS, Mak A. The socioeconomic burden of SLE. *Nat Rev Rheumatol*. 2009; 5:400–4. [PubMed: 19506585]
7. Petri M, Purvey S, Fang H, Magder LS. Predictors of organ damage in systemic lupus erythematosus: the Hopkins Lupus Cohort. *Arthritis Rheum*. 2012; 64:4021–8. [PubMed: 22932985]
8. Gonzalez LA, Toloza SM, McGwin G Jr, Alarcon GS. Ethnicity in systemic lupus erythematosus (SLE): its influence on susceptibility and outcomes. *Lupus*. 2013; 22:1214–24. [PubMed: 24097993]
9. Ugarte-Gil MF, Acevedo-Vasquez E, Alarcon GS, Pastor-Asurza CA, Alfaro-Lozano JL, Cucho-Venegas JM, et al. The number of flares patients experience impacts on damage accrual in systemic

- lupus erythematosus: data from a multiethnic Latin American cohort. *Ann Rheum Dis.* 2015; 74:1019–23. [PubMed: 24525909]
10. Bruce IN, O’Keeffe AG, Farewell V, Hanly JG, Manzi S, Su L, et al. Factors associated with damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort. *Ann Rheum Dis.* 2015; 74:1706–13. [PubMed: 24834926]
 11. Alarcon GS, McGwin G Jr, Petri M, Ramsey-Goldman R, Fessler BJ, Vila LM, et al. Time to renal disease and end-stage renal disease in PROFILE: a multiethnic lupus cohort. *PLoS Med.* 2006; 3:e396. [PubMed: 17076550]
 12. Sacks J, Helmick C, Langmaid G, Snizek J. From the Centers for Disease Control and Prevention. Trends in deaths from systemic lupus erythematosus--United States, 1979–1998. *JAMA.* 2002; 287:2649–50. [PubMed: 12035789]
 13. Gottschalk TA, Tsantikos E, Hibbs ML. Pathogenic Inflammation and Its Therapeutic Targeting in Systemic Lupus Erythematosus. *Frontiers in immunology.* 2015; 6:550. [PubMed: 26579125]
 14. Yao Y, Wang JB, Xin MM, Li H, Liu B, Wang LL, et al. Balance between inflammatory and regulatory cytokines in systemic lupus erythematosus. *Genet Mol Res.* 2016:15.
 15. Bauer JW, Baechler EC, Petri M, Batliwalla FM, Crawford D, Ortmann WA, et al. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. *PLoS Med.* 2006; 3:e491. [PubMed: 17177599]
 16. Davas EM, Tsirogianni A, Kappou I, Karamitsos D, Economidou I, Dantis PC. Serum IL-6, TNFalpha, p55 srTNFalpha, p75srTNFalpha, srIL-2alpha levels and disease activity in systemic lupus erythematosus. *Clin Rheumatol.* 1999; 18:17–22. [PubMed: 10088943]
 17. Morel J, Roubille C, Planelles L, Rocha C, Fernandez L, Lukas C, et al. Serum levels of tumour necrosis factor family members a proliferation-inducing ligand (APRIL) and B lymphocyte stimulator (BLyS) are inversely correlated in systemic lupus erythematosus. *Ann Rheum Dis.* 2009; 68:997–1002. [PubMed: 18676986]
 18. Segurado OG, Sasso EH. Vectra DA for the objective measurement of disease activity in patients with rheumatoid arthritis. *Clin Exp Rheumatol.* 2014; 32:S-29–34.
 19. Munroe ME, Vista ES, Guthridge JM, Thompson LF, Merrill JT, James JA. Pro-inflammatory adaptive cytokines and shed tumor necrosis factor receptors are elevated preceding systemic lupus erythematosus disease flare. *Arthritis & rheumatology.* 2014; 66:1888–99. [PubMed: 24578190]
 20. Crowe SR, Merrill JT, Vista ES, Dedek AB, Thompson DM, Stewart S, et al. Influenza vaccination responses in human systemic lupus erythematosus: impact of clinical and demographic features. *Arthritis Rheum.* 2011; 63:2396–406. [PubMed: 21598235]
 21. Stringer EA, Baker KS, Carroll IR, Montoya JG, Chu L, Maecker HT, et al. Daily cytokine fluctuations, driven by leptin, are associated with fatigue severity in chronic fatigue syndrome: evidence of inflammatory pathology. *Journal of translational medicine.* 2013; 11:93. [PubMed: 23570606]
 22. Dupont NC, Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. *J Reprod Immunol.* 2005; 66:175–91. [PubMed: 16029895]
 23. Dossus L, Becker S, Achaintre D, Kaaks R, Rinaldi S. Validity of multiplex-based assays for cytokine measurements in serum and plasma from “non-diseased” subjects: comparison with ELISA. *J Immunol Methods.* 2009; 350:125–32. [PubMed: 19748508]
 24. Bruner BF, Guthridge JM, Lu R, Vidal G, Kelly JA, Robertson JM, et al. Comparison of autoantibody specificities between traditional and bead-based assays in a large, diverse collection of patients with systemic lupus erythematosus and family members. *Arthritis Rheum.* 2012; 64:3677–86. [PubMed: 23112091]
 25. Hughes-Austin JM, Deane KD, Derber LA, Kolfenbach JR, Zerbe GO, Sokolove J, et al. Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: Studies of the Aetiology of Rheumatoid Arthritis (SERA). *Ann Rheum Dis.* 2013; 72:901–7. [PubMed: 22915618]
 26. Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* 2002; 13:323–40. [PubMed: 12220547]

27. Weckerle CE, Franek BS, Kelly JA, Kumabe M, Mikolaitis RA, Green SL, et al. Network analysis of associations between serum interferon-alpha activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum.* 2011; 63:1044–53. [PubMed: 21162028]
28. Ko K, Koldobskaya Y, Rosenzweig E, Niewold TB. Activation of the Interferon Pathway is Dependent Upon Autoantibodies in African-American SLE Patients, but Not in European-American SLE Patients. *Frontiers in immunology.* 2013; 4:309. [PubMed: 24101921]
29. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis & rheumatology.* 2014; 66:1583–95. [PubMed: 24644022]
30. Oriss TB, Krishnamoorthy N, Ray P, Ray A. Dendritic cell c-kit signaling and adaptive immunity: implications for the upper airways. *Current opinion in allergy and clinical immunology.* 2014; 14:7–12. [PubMed: 24300419]
31. Ray P, Krishnamoorthy N, Oriss TB, Ray A. Signaling of c-kit in dendritic cells influences adaptive immunity. *Ann N Y Acad Sci.* 2010; 1183:104–22. [PubMed: 20146711]
32. Ray P, Krishnamoorthy N, Ray A. Emerging functions of c-kit and its ligand stem cell factor in dendritic cells: regulators of T cell differentiation. *Cell Cycle.* 2008; 7:2826–32. [PubMed: 18787413]
33. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *Journal of autoimmunity.* 2008; 31:252–6. [PubMed: 18502610]
34. Scheller J, Chalaris A, Garbers C, Rose-John S. ADAM17: a molecular switch to control inflammation and tissue regeneration. *Trends Immunol.* 2011; 32:380–7. [PubMed: 21752713]
35. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2011; 63:3918–30. [PubMed: 22127708]
36. Wallace DJ, Stohl W, Furie RA, Lisse JR, McKay JD, Merrill JT, et al. A phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus. *Arthritis Rheum.* 2009; 61:1168–78. [PubMed: 19714604]
37. Cogollo E, Silva MA, Isenberg D. Profile of atacicept and its potential in the treatment of systemic lupus erythematosus. *Drug design, development and therapy.* 2015; 9:1331–9.
38. Gordon C, Wofsy D, Wax S, Li Y, Pena Rossi C, Isenberg D. Post-hoc analysis of the Phase II/III APRIL-SLE study: Association between response to atacicept and serum biomarkers including BlyS and APRIL. *Arthritis & rheumatology.* 2016
39. Tokano Y, Morimoto S, Kaneko H, Amano H, Nozawa K, Takasaki Y, et al. Levels of IL-12 in the sera of patients with systemic lupus erythematosus (SLE)--relation to Th1- and Th2-derived cytokines. *Clin Exp Immunol.* 1999; 116:169–73. [PubMed: 10209522]
40. Gomez D, Correa PA, Gomez LM, Cadena J, Molina JF, Anaya JM. Th1/Th2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? *Semin Arthritis Rheum.* 2004; 33:404–13. [PubMed: 15190525]
41. Mok MY, Wu HJ, Lo Y, Lau CS. The Relation of Interleukin 17 (IL-17) and IL-23 to Th1/Th2 Cytokines and Disease Activity in Systemic Lupus Erythematosus. *J Rheumatol.* 2010
42. Liu CC, Ahearn JM. The search for lupus biomarkers. *Best practice & research. Clinical rheumatology.* 2009; 23:507–23. [PubMed: 19591781]
43. Petri MA, van Vollenhoven RF, Buyon J, Levy RA, Navarra SV, Cervera R, et al. Baseline Predictors of Systemic Lupus Erythematosus Flares: Data From the Combined Placebo Groups in the Phase III Belimumab Trials. *Arthritis Rheum.* 2013; 65:2143–53. [PubMed: 23754628]
44. Villegas-Zambrano N, Martinez-Taboada VM, Bolivar A, San Martin M, Alvarez L, Marin MJ, et al. Correlation between clinical activity and serological markers in a wide cohort of patients with systemic lupus erythematosus: an eight-year prospective study. *Ann N Y Acad Sci.* 2009; 1173:60–6. [PubMed: 19758133]
45. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, et al. Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell.* 2016; 165:1548–50. [PubMed: 27259156]

46. Curtis JR, van der Helm-van Mil AH, Knevel R, Huizinga TW, Haney DJ, Shen Y, et al. Validation of a novel multibiomarker test to assess rheumatoid arthritis disease activity. *Arthritis care & research*. 2012; 64:1794–803. [PubMed: 22736476]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Multiple immune pathways are dysregulated prior to disease flare.
- Baseline soluble mediator levels correlate with future disease activity at flare.
- A soluble mediator score signals impending SLE flare in African-American patients.

Figure 1.

Soluble mediator levels are altered in African-American (AA) SLE patients with impending disease flare vs. non-flare AA SLE patients. Baseline levels of plasma soluble mediators were assayed in 13 AA SLE patients who experienced disease flare 6–12 weeks post baseline assessment (Flare) vs. 13 demographically matched SLE patients who did not experience a flare (NF). Examined factors included (A) innate mediators, (B) Th1-type mediators, (C) Th17-type mediators, (D) regulatory mediators, (E) IFN-associated chemokines, (F) TNF superfamily, and (G) SCF. Levels are presented as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ by Wilcoxon's matched pairs test.

Figure 2.

Soluble mediator levels are altered in African-American (AA) SLE patients with impending disease flare vs. comparable non-flare period. Baseline levels of plasma soluble mediators were assayed in 18 AA SLE patients who experienced disease flare 6–12 weeks post baseline assessment (Flare) vs. comparable non-flare period in the same SLE patients (SNF). Examined factors included (A) innate mediators, (B) Th1-type mediators, (C) Th17-type mediators, (D) regulatory mediators, (E) IFN-associated chemokines, (F) TNF superfamily, and (G) SCF. Levels are presented as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by Wilcoxon's matched pairs test.

Figure 3.

Baseline soluble mediator score, but not baseline clinical disease activity, differentiates African-American (AA) SLE patients with impending disease flare. Baseline SELENA-SLEDAI scores were determined for (A) 13 AA SLE patients who experienced disease flare 6–12 weeks post baseline assessment (Flare) versus 13 race, gender, and age (± 5 years) matched SLE patients with no flare over the 6–12 week follow-up period (non-flare; NF) or (B) scores from 18 AA SLE patients who experienced disease flare 6–12 weeks post baseline assessment (Flare) versus the same patient in year without disease flare (self non-flare; SNF). The soluble mediator score was also calculated for (C) Flare versus NF patients or (D) Flare versus SNF periods. **** $p < 0.0001$ by Wilcoxon's matched pairs test.

Table 1

Demographics and clinical characteristics of African-American (AA) SLE patients

	Flare (n = 13)	NF ^a (n = 13)	p-value	Flare (n = 18)	SNF ^b (n = 18)	p-value
Age, mean ± SD years ^c	40.9 ± 10.5	42.7 ± 12.4	0.3093	40.5 ± 12.1	40.5 ± 12.6	-
Medications: n positive (%)						
Prednisone ^d	6 (46.1%)	7 (53.8%)	1.0000	11 (61.1%)	10 (55.6%)	1.0000
Immunosuppressants ^{d,e}	4 (30.8%)	4 (30.8%)	1.0000	6 (33.3%)	9 (50%)	0.4998
Hydroxychloroquine ^d	5 (38.5%)	9 (69.2%)	0.2377	14 (77.8%)	9 (50%)	0.1642
Baseline						
Baseline autoantibody specificities: n positive (%)						
Anti-dsDNA ^d	5 (38.5%)	3 (23.1%)	0.6728	6 (33.3%)	2 (11.1%)	0.2285
Anti-chromatin ^d	6 (46.2%)	3 (23.1%)	0.4110	5 (27.8%)	5 (27.8%)	1.0000
Anti-Ro/SSA ^d	2 (15.4%)	2 (15.4%)	1.0000	7 (38.9%)	5 (27.8%)	0.7247
Anti-La/SSB ^d	1 (7.7%)	1 (7.7%)	1.0000	2 (11.1%)	1 (5.6%)	1.0000
Anti-Sm ^d	5 (38.5%)	3 (23.1%)	0.6728	3 (16.7%)	3 (16.7%)	1.0000
Anti-SmRNP ^d	6 (46.2%)	4 (30.8%)	0.6882	8 (44.4%)	8 (44.4%)	1.0000
Anti-RNP ^d	4 (30.8%)	2 (15.4%)	0.6447	5 (27.8%)	6 (33.3%)	1.0000
Baseline # of autoantibody specificities: mean ± SD ^f	2.2 ± 2.2	1.4 ± 1.9	0.3711	2.0 ± 2.1	1.7 ± 1.7	0.4609
Baseline ESR: mean ± SD mm/hour ^f	38.0 ± 18.7	25.7 ± 17.4	0.2100	33.7 ± 18.0	28.6 ± 21.9	0.4609
Follow-up						
SELENA-SLEDAI score (at follow-up): mean ± SD ^c	7.3 ± 2.9	2.9 ± 3.0	0.0002	8.4 ± 6.2	4.1 ± 3.7	0.0020
SELENA-SLEDAI organ system manifestations (at follow-up): n positive (%) ^d	12 (92.3%)	6 (46.2%)	0.0302	17 (94.4%)	10 (55.6%)	0.0178
CNS ^d	0	0	--	1 (5.6%)	0	1.0000
Arthritis ^d	8 (61.5%)	5 (38.5%)	0.4338	14 (77.8%)	8 (44.4%)	0.0858
Renal ^d	1 (7.7%)	0	1.0000	0	1 (5.6%)	1.0000
Mucocutaneous ^d	8 (61.5%)	2 (15.4%)	0.0414	10 (55.6%)	4 (22.2%)	0.0858

	Flare (n = 13)	NF ^a (n = 13)	p-value	Flare (n = 18)	SNF ^b (n = 18)	p-value
Serositis ^d	3 (23.1%)	1 (7.7%)	0.5930	3 (16.7%)	1 (5.6%)	0.6026
Hematologic ^d	0	0	--	1 (5.6%)	1 (5.6%)	1.0000

^a AA SLE patients with impending SELENA-SLEDAI defined disease flare at follow-up (6 or 12 weeks after baseline) vs. race, gender, and age ($\pm$ 5 years) matched SLE patients who did not experience disease flare over the same time period (non-flare; NF)

^b AA SLE patients with impending disease SELENA-SLEDAI defined disease flare at follow-up (6 or 12 weeks after baseline) vs. the *same* SLE patients during a year of the study without disease flare (self non-flare; SNF)

^c Statistical significance determined by paired t-test

^d Statistical significance determined by Fisher's exact test; *p* 0.05

^e Immunosuppressants = azathioprine, mycophenolate mofetil, cyclophosphamide

^f Statistical significance determined by Wilcoxon's matched pairs test

^g Sum of ranks (Bmax, Ka, HA Inhibition)

Table 2

Baseline soluble mediators correlate with SELENA-SLEDAI score at follow-up (FU) evaluation and significantly contribute to the Soluble Mediator Score (SMS) in flare vs. non-flare (NF) SLE patients

Pathway	Soluble mediator	Correlation with FU SELENA-SLEDAI Score			Contribution to SMS		
		Spearman r	p-value	q-value ^a	OR ^b	P value ^c	q-value ^a
Innate	IL-1 α	0.4858	0.0119	0.0385	18.3	0.0048	0.0159
	IFN- β	0.4262	0.0299	0.0691	57.0	0.0005	0.0044
Th1-like	IL-12p70	0.5083	0.0080	0.0324	57.0	0.0005	0.0044
	IL-2R α	0.4143	0.0354	0.0697	66.0	0.0002	0.0027
Th17-like	IL-6	0.4108	0.0371	0.0697	47.7	0.0016	0.0085
	IL-17A	0.5810	0.0019	0.0178	225.0	<0.0001	0.0018
	IL-21	0.5164	0.0069	0.0319	17.5	0.0391	0.0901
Regulatory	TGF- β	-0.5936	0.0014	0.0178	41.7	0.0016	0.0085
	MCP-1/CCL2	0.4524	0.0203	0.0547	7.5	0.0472	0.1001
	MCP-3/CCL7	0.5737	0.0022	0.0178	18.3	0.0048	0.0159
Chemokine/Adhesion molecules	MIP-1 β /CCL4	0.4076	0.0388	0.0697	5.3	0.1107	0.1778
	IL-8/CXCL8	0.5533	0.0034	0.0183	27.0	0.0036	0.0147
	MIG/CXCL9	0.4680	0.0159	0.0468	6.4	0.0968	0.1769
	IP-10/CXCL10	0.4264	0.0298	0.0691	11.1	0.0169	0.0427
TNF superfamily	ICAM-1	0.5605	0.0029	0.0183	5.3	0.1107	0.1778
	CD154/CD40L	0.6164	0.0008	0.0178	144.0	<0.0001	0.0018
	TRAIL	0.5020	0.0090	0.0324	12.4	0.0154	0.0408
Other	SCF	0.4158	0.0346	0.0346	12.4	0.0154	0.0408

^a Adjusted p-values based on False Discovery Rate of 52 mediators assessed; **q 0.05**

^b Odds Ratio (# of Flare vs NF subjects with positive or negative contribution to SMS)

^c Statistical significance determined by Fisher's exact test

Table 3
Association between Soluble Mediator Score and SLE Disease Activity in AA Patients

Soluble Mediator Score					
	Median	SD	p value ^a	OR ^b	95% CI
Flare subjects (n = 13)	7.41	4.73	< 0.0001	729	13.4 to 13518
NF subjects (n = 13)	-8.46	2.36			
Flare subjects (n = 18)	4.09	2.61	< 0.0001	164	7.8 to 3425
SNF subjects (n = 18)	-4.01	2.81			

^aStatistical significance determined by Wilcoxon matched pairs test

^bOdds Ratio (# of Flare vs NF [or SNF] subjects with positive or negative soluble mediator score [SMS])

^cStatistical significance determined by Fisher's exact test