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MEETING ABSTRACTS Open Access

Lupus 2012: New targets, new approaches

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MEETING ABSTRACTS

A1

Epigenetics and lupus

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A1

Lupus develops when genetically predisposed people encounter environmental agents that initiate flares. Current evidence indicates that the environmental contribution is mediated by T-cell DNA demethylation. DNA methylation patterns are established during differentiation, and silence inappropriate or unnecessary genes by promoting a condensed chromatin configuration that is inaccessible to transcription factors. The methylation patterns are then replicated each time a cell divides by DNA methyltransferase 1 (Dnmt1). Dnmt1 is upregulated during mitosis, binds the replication fork, and catalyzes transfer of the methyl group from S-adenosylmethionine (SAM) to dC bases in the daughter DNA strand only where the parent strand is methylated. Environmental agents that block ERK pathway signaling prevent Dnmt1 upregulation, and low Dnmt1 levels synergize with dietary micronutrient deficiencies that decrease SAM pools to impair methylation of the daughter strand. This activates genes silenced only by DNA methylation.

Inhibiting T-cell DNA methylation converts helper $CD4^+$ T cells into autoreactive, cytotoxic, proinflammatory cells that cause lupus-like autoimmunity in mice. Similar changes in $CD4^+$ T-cell DNA methylation and gene expression are found in patients with active lupus. Procainamide and hydralazine, which cause ANAs in a majority of patients and lupus in a genetically predisposed subset, also inhibit T-cell DNA methylation. The lupus T-cell DNA methylation defect has been traced to low Dnmt1 levels caused by decreased ERK pathway signaling, and the signaling defect has now been traced to PKCδ inactivation caused by oxidative damage.

The importance of decreased ERK pathway signaling was confirmed by generating a transgenic mouse with an inducible dominant negative MEK. Inducing the signaling defect selectively in T cells decreases Dnmt1, causing anti-DNA antibodies in mice without lupus genes, and higher anti-DNA antibody levels and an immune complex glomerulonephritis in mice with lupus genes. Autoantibody levels and kidney disease are suppressed by dietary transmethylation micronutrient supplementation in these mice.

Epigenetic mechanisms also contribute to the gender dimorphism in lupus. Immune genes on the normally silenced X chromosome demethylate in women with active lupus, contributing to flare severity. In contrast, men with only one X chromosome require a greater genetic predisposition and/or greater degree of DNA demethylation to develop a lupus flare equal in severity to women.

Together, these studies indicate that environmental agents including oxidative stress and diet combine to inhibit T-cell DNA methylation, and that the epigenetically modified cells cause lupus-like autoimmunity in genetically predisposed people and mice.

A2

Follicular helper T Cells and the B cells they help

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A2

Background: CD4 T cells help B cells produce antibodies following antigen challenge. This response classically occurs in germinal centers (GC) located in B-cell follicles of secondary lymphoid organs (SLO), a site of immunoglobulin isotype switching and affinity maturation. GC formation requires specialized CD4 T cells, T-follicular helper (Tfh) cells, which localize to follicles and provide B cells with survival and differentiation signals that are essential for B-cell maturation into memory and long-lived plasma cells. Pathogenic autoantibodies in human and murine lupus arise in a like manner. Although Tfh cells are critical for GC development, their genesis in humans, role in promotion of autoimmunity, and potential as therapeutic targets in SLE are incompletely understood. To address these issues, we dissected Tfh cell development and function, defining their transcriptional regulation, migration, and function *in vivo* in normal and lupus-prone mice and *ex vivo* in normal humans and patients with SLE.

Methods: We used a combination of approaches - flow cytometry, confocal microscopy, microarrays, quantitative chromatin immunoprecipitation and DNA sequencing (ChIP-seq), retroviral overexpression, and T-cell-B-cell helper assays - to characterize Tfh cells in normal mice and in lupus-prone strains, and from the tonsils of normal humans and the blood of patients with SLE.

Results: We found that the transcription factor Bcl6 (B-cell CLL/lymphoma 6) is necessary and sufficient for Tfh development and function, via genetic control of Tfh proteins that are essential for their migration to B-cell follicles and GC and subsequent B-cell maturation. We dissected steps in Tfh development within SLO, beginning with their genesis in the T-cell zone followed by emigration to sites of B-cell interaction outside the B-cell follicle, where we have shown that B cells serve to provide signals for continued Tfh expansion and follicular migration. We have now begun to tease apart the factors that mediate T-cell-B-cell collaboration in the follicle; these represent therapeutic targets in SLE. Finally, we have shown that patients with SLE have expansion of Tfh cells in the blood, a finding that highlights their potential role in the pathogenesis of SLE and as likely therapeutic targets.

Conclusion: These studies help define the developmental pathways for Tfh cells, and the steps that enable these cells to function in the B-cell follicle to promote immunoglobulin and autoantibody production. They have also helped define markers of Tfh cells in normals and autoimmune individuals, and suggest that they are a promising therapeutic target in patients.

A3

Longitudinal analysis of mRNA transcripts and plasma proteins to define a biomarker associated with lupus disease activity

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A3

Objective: Lupus, a chronic autoimmune disease, is characterized by a variable clinical course, with periods of active disease. Identification of a biomarker or biomarker panel associated with clinical disease activity would be useful for disease management, assessment of response to therapeutic intervention in practice or clinical trials, and might suggest cellular or molecular targets for future therapies. To identify biomarkers that reflect lupus disease activity, we assessed longitudinal clinical, gene expression and proteomic data from SLE patients.
Methods: One hundred and sixty-nine RNA extracts from PBMC and plasma samples were collected longitudinally (up to 3 years) from 23 SLE patients and five healthy donors (HD), and SLEDAI and BILAG scores were recorded. All SLE patients fulfilled ACR criteria for the disease. PBMC mRNA profiles for each visit were established using Affymetrix GeneChips. A panel of proinflammatory cytokines was evaluated using Multi-Analyte Profiling technology (Rules-Based Medicine, Austin, TX, USA). Longitudinal data analysis was performed using R (R Development Core Team) and the R packages lme4 and languageR. Data were analyzed using linear mixed-effects (LME) models.
Results: K-mean cluster analysis was first used to identify groups of gene transcripts that fluctuate in relation to disease activity, and representative transcripts were selected from each cluster. Thirteen plasma factors were identified as significantly increased in SLE patients compared with HD, and 14 plasma factors were significantly associated with disease activity. LME analysis was applied to the dataset to identify those transcripts and plasma factors that best define clinical disease activity. Statistical correlation with disease activity for this biomarker panel was compared with traditional measures of disease activity.
Conclusion: A combination of mRNA transcripts and plasma factors, when assessed as a panel, shows a high correlation with clinical disease activity in patients with SLE. Validation of this biomarker panel in an extended patient group may provide support for measurement of these transcripts and proteins as an informative correlate of disease activity and a tool for patient management.

A4

New therapies

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A4

The past decade has brought unprecedented progress in the refinement of conventional therapies for systemic lupus erythematosus (SLE) and the development of biologic therapies for SLE. Extensive recent evidence has expanded our understanding of the potential benefits of antimalarial therapy and has demonstrated improved approaches to the use of cyclophosphamide. Concurrently, a strong foundation of evidence has been generated to support the use of mycophenolate mofetil, especially in lupus nephritis.
Against this background, there has been mounting excitement surrounding the promise of biologic therapies. Belimumab demonstrated efficacy in two phase III trials involving patients with diverse, nonrenal non-CNS, manifestations of SLE. The success of these trials has drawn attention to a novel primary endpoint, the SLE Responder Index (SRI). In this regard, it bears emphasizing that the trials were positive because the drug had a demonstrable effect, not because of the novelty of the endpoint. Indeed, the SLEDAI component of the outcome measure distinguished the treatment groups from the control groups with virtually the same statistical certainty as the SRI. Therefore, it remains to be determined which, if either, of these outcome measures might perform best in future lupus trials. At present, numerous follow-up trials are underway to assess which patient subpopulations and which disease manifestations are responsive to belimumab.
At the same time that belimumab was being tested in patients with lupus manifestations other than nephritis, abatacept was being tested in patients with lupus nephritis. A large international trial failed to achieve its primary endpoint. However, *post-hoc* analyses have raised questions about that result. Specifically, when the data from the abatacept trial were subjected to the outcome measures from other major lupus nephritis trials (LUNAR and ALMS), the complete response (CR) rates among subjects treated with abatacept were substantially higher than the CR rates in the control group [1]. Subsequent analyses of the data from this trial examined the discriminatory capability of other possible outcome measures and demonstrated that, at least in this dataset, the CR rate at 12 months discriminated treatment from control groups more effectively than other common outcome measures (including partial response (PR), overall response (CR+PR), treatment failure rate, or response rates at 6 months rather than 12 months). Studies currently in progress should help to clarify whether abatacept is effective in the treatment of lupus nephritis.

Reference

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GENETICS/EPIDEMIOLOGY

A5

MicroRNA-3148 modulates differential gene expression of the SLE-associated *TLR7* variant

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Background: We identified the G allele of *TLR7* 3'-UTR SNP (rs3853839) associated with increased *TLR7* transcripts, a more pronounced IFN signature and risk for SLE in 9,274 Eastern Asians ($P_{combined} = 6.5 \times 10^{-10}$) [1]. The current study sought replication of SLE-associated SNP(s) in non-Asian ancestries and explored molecular mechanisms underlying an identified gene variant that affects TLR7 expression.
Methods: We conducted genotyping, imputation and association for 98 to 116 SNPs (varying among different ancestries) covering 80 kb of *TLR7-TLR8* in European Americans (EA), African Americans (AA) and Hispanics enriched for the Amerindian-European admixture (HS). Haplotype-based conditional testing was conducted to distinguish independent association signals. Mantel-Haenszel testing was used in transancestral meta-analysis. Association of genotypes with TLR7 expression was examined using RT-PCR, flow cytometry and reporter assays. Pyrosequencing was used to measure allelic variations in *TLR7* transcript levels.
Results: The rs3853839 was confirmed as the only variant within *TLR7-TLR8* exhibiting consistent and independent association with SLE in our transancestral fine-mapping ($P_{meta} = 7.5 \times 10^{-11}$, OR (95% CI) = 1.24 (1.18 to 1.34)) in 13,339 subjects of EA (3,936 cases vs. 3,491 controls), AA (1,679 vs. 1,934) and HS (1,492 vs. 807) ancestries. PBMCs from normal G-allele carriers exhibited elevated levels of *TLR7* mRNA ($P = 0.01$ in men and $P = 0.02$ in

women) and protein ($P = 0.009$ in men and $P = 0.038$ in women). PBMCs from heterozygotes exhibited higher G/C allele ratios of *TLR7* transcripts 4 hours after incubation with actinomycin D (inhibitor of transcription initiation) ($P = 0.04$), indicating slower degradation of G allele-containing transcript. The nonrisk allele, but not the risk allele, was predicted to match microRNA-3148 (miR-3148) at the second base in the binding site. Transcript levels of miR-3148 and TLR7 were inversely correlated in PBMCs from 16 SLE patients and 21 controls ($R^2 = 0.255$, $P = 0.001$), suggesting miR-3148 modulating TLR7 expression. Overexpression of miR-3148 via transfection into HEK 293 cells led to a more than twofold reduction in luciferase activity driven by the *TLR7* 3′-UTR segment containing the nonrisk allele than that containing the risk allele ($P = 0.001$).

Conclusion: We identified and confirmed a genome-wide significant association between rs3853839 and SLE susceptibility in 22,613 subjects of Eastern Asian, EA, AA and HS ancestries ($P_{meta} = 6.4 \times 10^{-19}$, OR (95% CI) = 1.26 (1.20 to 1.32)). Reduced modulation by miR-3148 confers slower degradation of the risk allele containing *TLR7* transcript, resulting in elevated levels of gene products and a more robust type I IFN signature.

Reference

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A6

Differential DNA methylation associated with anti-dsDNA autoantibody production in systemic lupus erythematosus

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A6

Background: Aberrant DNA methylation has been implicated in the pathogenesis of systemic lupus erythematosus (SLE), with less DNA methylation observed in SLE patients compared with healthy controls. We sought to determine whether DNA methylation differences are also associated with anti-dsDNA autoantibody production in SLE.

Methods: Genomic DNA from peripheral blood leukocytes was isolated from 326 SLE patients, including 158 anti-dsDNA-positive and 168 anti-dsDNA-negative. All SLE cases were female, of European descent, and had never smoked. DNA methylation profiles were initially characterized on a subset of patients ($n = 208$, with dsDNA-positive cases matched to dsDNA-negative cases on age and disease duration) using the Illumina HumanMethylation27 Beadchip (27 k chip). Subsequently, all patients ($n = 326$) were characterized for the HumanMethylation450 array, and analyses focused on the most strongly associated regions from the 27 k methylation data. Paired *t* tests were used to identify site-specific methylation differences associated with anti-dsDNA autoantibody production in the initial phase, with $P < 1.8 \times 10^{-6}$ (Bonferroni corrected) considered statistically significant. Genome regions that demonstrated statistically significant associations with dsDNA status were further assessed using the 450 k methylation data.

Results: Overall, less methylation was observed in anti-dsDNA positive patients compared with dsDNA-negative patients. Analysis of the 27 k methylation data demonstrated decreased methylation of a CpG site near *SOCS2* ($P = 3.5 \times 10^{-7}$), an inhibitor of the STAT family of transcription factors, as well as a site near *GGT1* ($P = 4.3 \times 10^{-3}$). In addition, increased methylation of two CpG sites in *PRIC285*, a transcriptional co-activator for nuclear receptors, was significantly associated with anti-dsDNA autoantibody production ($P = 1.9 \times 10^{-7}$ and 3.4×10^{-7}). Subsequent analysis of 114 methylation sites from the 450 k methylation data in these regions demonstrated very strong association with the methylation site cg22764925, located in the 5′-UTR region of *GGT1* ($P = 2.8 \times 10^{-11}$), with an average difference of 7.7% between methylation levels of anti-dsDNA-positive and anti-dsDNA-negative patients. The methylation site cg11738543, and several other CpG sites in the *SOCS2* gene, were also associated with anti-dsDNA antibody status ($P = 4.2 \times 10^{-8}$).

Conclusion: These findings suggest that abnormal methylation patterns of specific genes, including *GGT1* and *SOCS2*, may contribute to autoantibody production in SLE. Studies of DNA methylation and other epigenetic modifications may help elucidate biologic mechanisms involved in the pathogenesis of SLE.

A7

Area-level socioeconomic status and variation in medication use among Medicaid enrollees with incident systemic lupus erythematosus, 2000 to 2004

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Background: Differences in access to medications may be related to socioeconomic disparities in outcomes among individuals with systemic lupus erythematosus (SLE). We investigated county-level socioeconomic status (SES) and corticosteroid, hydroxychloroquine and immunosuppressant use among individuals in the low-income US Medicaid population.

Methods: From the US Medicaid Analytic eXtract (MAX) data, containing all billing claims from 2000 to 2004, we identified adults, aged 18 to 65, with incident SLE (≥3 SLE ICD-9 codes (710.0), >30 days apart, with 24 months of prior enrollment without SLE claims). Sex, age, race/ethnicity, ZIP code, and geographic region were obtained. We determined health professional shortage areas, number of state-level rheumatologists, and county-level SES using a validated composite of US Census variables. Our outcome measures were use at any time of corticosteroids, hydroxychloroquine, or an immunosuppressant (azathioprine, mycophenolate mofetil, cyclophosphamide, cyclosporine or tacrolimus). We used multivariable logistic regression to examine relationships between sociodemographic variables and medication prescriptions.

Results: Of 3,965 Medicaid enrollees with incident SLE, the mean age was 39.5 ± 12; 94.1% were female, 41.2% African American, 33.7% White, and 15.2% Hispanic. A total of 67.4% received corticosteroids, 49.5% received hydroxychloroquine, and 17.5% received an immunosuppressant. Adjusting for months of enrollment, 77.5% of patients in the lowest SES quartile received corticosteroids compared with 65.9% in the highest (OR = 1.8, 95% CI = 1.27 to 2.58). In our fully adjusted model (Table 1), increasing county-level SES among Medicaid enrollees with incident SLE was associated with a lower odds of receiving a corticosteroid prescription, but a higher odds of receiving an immunosuppressant prescription. Hydroxychloroquine use did not vary significantly by SES.

Conclusion: In higher SES areas, corticosteroid use among patients with incident SLE appears to be less frequent, while immunosuppressant use

Table 1(abstract A7) Medication prescriptions among Medicaid enrollees with incident SLE, 2000 to 2004 according to county-level SES[a]

	Corticosteroids		Hydroxychloroquine		Immunosuppressants[b]	
	OR	95% CI	OR	95% CI	OR	95% CI
Increasing county-level SES	0.94	0.89-0.99	1.04	0.99-1.09	1.12	1.05-1.20

[a]Multivariable logistic regression models adjusted for number of Medicaid-enrolled months, age at first SLE claim, race/ethnicity, sex, geographic region, Health Professional Shortage Area, and state-level number of rheumatologists. [b]Immunosuppressants include: azathioprine, mycophenolate mofetil, cyclophosphamide (oral and intravenous), cyclosporine or tacrolimus.

may be more frequent. Further studies are necessary to determine whether medication differences contribute to disparities in outcomes.

A8

SLE risk alleles and cell development and activation

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A8

Understanding the functionality of lupus susceptibility alleles may best be done using cells of healthy individuals harboring one or two copies of the susceptibility allele. With this approach, confounding variables introduced by disease and medication are avoided. We have studied the SLE susceptibility allele of Blimp-1 in dendritic cells and Csk in B cells. The Blimp-1 risk allele exhibits low expression and leads to an increased secretion of proinflammatory cytokines. The Csk risk allele leads to enhanced expression and an increased response to B-cell receptor signaling. These effects of the risk alleles on activated or resting cells, respectively, provide insight into disease pathogenesis.

A9

Genetic-epigenetic interaction in lupus

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A9

Systemic lupus erythematosus is a genetically complex autoimmune disease. A large body of evidence suggests an important role for epigenetic variation, particularly DNA methylation changes, in the pathogenesis of lupus. We recently performed a comprehensive evaluation of the DNA methylome in lupus T cells and identified a number of differentially methylated loci that can contribute to the pathogenesis of the disease. By analyzing the interaction between genetic risk, T-cell DNA demethylation, and the SLEDAI scores, we demonstrated that the (genetic risk)/(T-cell DNA methylation) ratio increased directly with disease activity in lupus patients. Furthermore, men with lupus require a higher genetic risk and/or lower T-cell DNA methylation levels to achieve a lupus flare of equal severity to women, suggesting genetic-epigenetic interaction in explaining the sex bias in lupus. We have also established the genetic region containing methyl-CpG-binding protein 2 (*MECP2*) as a lupus susceptibility locus. MeCp-2 is a key transcription factor critically involved in regulating the expression of methylation-sensitive genes, and directly recruits DNA methyltransferase 1 (DNMT1). Indeed, recent data from our group demonstrate that the lupus-associated variants in *MECP2* induce DNA methylation changes in key inflammatory genes. Our data suggest that efforts to study genetic-epigenetic interactions in lupus will further our understanding of the disease pathogenesis and might help to explain, at least in part, the missing heritability in lupus.

A10

Serum chemokine levels predict flares of disease activity in two independent systemic lupus erythematosus cohorts

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A10

Background: Gene expression profiling of blood samples from SLE patients has revealed an interferon signature defined by increased expression of type I interferon (IFN)-inducible genes. In lupus patients with quiescent disease, we previously showed that elevated serum levels of IFN-inducible chemokines IP-10, MCP-1, and MIP-3b identified a subgroup of patients who were more likely to flare within the following year. The goal of this study was to derive flare risk definitions from the discovery cohort and test those definitions in an independent patient group.
Methods: Consenting SLE patients were enrolled in the Autoimmune Biomarkers Collaborative Network (ABCoN) study from the Hopkins Lupus Cohort. Sera were isolated from blood collected in serum-separator tubes. SearchLight multiplexed immunoassays (Aushon Biosystems) were used to quantitate serum levels of IP-10, I-TAC, MCP-1, and MIP-3b. ABCoN Cohort 1 was used for discovery of significant variables for defining flare risk and nonrisk, and those risk definitions were tested in the independent Cohort 2. In 254 patients from Cohort 1 with inactive or mild disease (SLEDAI ≤4) at baseline, we tested serum chemokine levels, the IFN gene score, and clinical laboratory values. Risk definitions were assessed by Kaplan-Meier survival analysis and multivariate Cox regression. Statistically significant markers derived from Cohort 1 were tested in Cohort 2, in which 262 patients had SLEDAI ≤4 at their first study visit.
Results: In both cohorts, patients with high chemokine scores (calculated from IP-10, MCP-1, and MIP-3b levels) had an increased frequency of future flare (Cohort 1, Kaplan-Meier $P = 0.0001$; Cohort 2, $P = 0.003$). In Cohort 1, individual markers IP-10, I-TAC, MIP-3b, and the IFN gene score identified patients who were more likely to flare (Kaplan-Meier $P < 0.05$). Risk definitions based on IP-10, I-TAC, and the IFN gene score were also significant in Cohort 2. In multivariate regression analysis, IP-10 and I-TAC were identified as significant predictors of flare (Cohort 1, $P = 0.003$; Cohort 2, $P = 0.04$).
Conclusion: Measurement of chemokine levels in frozen samples, collected from two longitudinally followed cohorts of SLE patients, identified several chemokines that were associated with development of flare in the subsequent 12-month period of time. A combination of chemokine levels may be used to predict flare risk in SLE and to aid in patient management.
Competing interests: EBC is entitled to receive royalties under a licensing agreement between LabCorp and the University of Minnesota.

CHALLENGES IN CLINICAL LUPUS

A11

Major congenital anomalies in children born to women with systemic lupus erythematosus

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A11

Background: Systemic lupus erythematosus (SLE) can cause considerable morbidity during pregnancy. Although several studies have evaluated fetal outcome in lupus pregnancy, only one small study assessed congenital anomalies in 30 children born to SLE mothers, showing no excess risk. In a large population-based study, we aimed to determine whether children born to women with SLE have an increased risk of major congenital anomalies compared with children born to women without SLE.
Methods: We identified all women who had ≥1 hospitalization for a delivery after SLE diagnosis using Quebec's physician billing and hospitalization databases (1989 to 2009). Women were defined as SLE cases if they had any of the following: ≥1 hospitalization with a diagnosis of SLE prior to the delivery; a diagnosis of SLE recorded at the time of their hospitalization for delivery; or ≥2 physician visits with a diagnosis of SLE, occurring 2 to 24 months apart, prior to the delivery. We randomly selected a general population control group, composed of women matched at least 4:1 for age and year of delivery, who did not have a diagnosis of SLE prior to or at the time of delivery. We identified children born live to SLE cases and their matched controls, and obtained information on all physician visits and hospitalizations incurred by these children. We ascertained major congenital anomalies, as defined by ≥1 hospitalization or physician visit with an ICD-9/10 diagnostic code for major congenital anomaly, within the first 12 months of life. We performed multivariate analyses to adjust for maternal demographics (that is, age, education, marital status), sex and birth order of child, major maternal co-morbidities (that is, pre-gestational diabetes, hypertension, asthma, depression), and relevant maternal medications (that is, antimalarials, corticosteroids, immunosuppressants).
Results: A total of 507 women with SLE had 721 children, while 5,862 matched controls had 8,561 children. Compared with controls, children born to women with SLE experienced slightly more major congenital anomalies (13.6% (95% CI = 11.3 to 16.3) vs. 10.4% (95% CI = 9.7 to 11.1)). In multivariate analyses, children born to women with SLE had a substantially increased risk of major congenital anomalies (adjusted OR = 1.28, 95%

CI = 1.01 to 1.62) compared with controls. Medication exposures did not seem to mediate the risk of major congenital anomalies.
Conclusion: Our findings suggest that, compared with children from the general population, children born to women with SLE have an increased risk of major congenital anomalies, and prompt further research to elucidate this issue.

A12

Contemporary estimates of the risk of end-stage renal disease in the first decade of proliferative lupus nephritis

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A12

Background: End-stage renal disease (ESRD) is a major cause of morbidity and costs in patients with systemic lupus erythematosus (SLE). Patients with proliferative lupus nephritis are at greatest risk of ESRD, but with recent treatment advances, the proportion of patients with proliferative nephritis who develop ESRD may be decreasing. We sought to learn the risk of ESRD in patients with proliferative lupus nephritis enrolled in studies since 1990.
Methods: In a systematic literature review, we searched PubMed, Embase, and the Cochrane Database of Systematic Reviews from inception to November 2011 to identify published articles on the risk of ESRD in lupus nephritis. We also searched references of retrieved articles and reviews. We excluded articles with fewer than 10 patients, less than 1 year of follow-up, those primarily of children, and those that did not report ESRD (dialysis or renal transplantation) as a specific outcome. Of 1,144 unique articles from the searches, we did a full-text review of 373 articles. One hundred and fifty-five articles met inclusion criteria, reported relevant data and were not duplicate reports on the same cohort. Thirty-one studies began enrollment in 1990 or later. Here we examined the 14 studies (15 arms) that reported outcomes of patients with proliferative lupus nephritis. We computed weighted averages of the proportion with ESRD at the mean follow-up. For studies with more than one treatment arm, we pooled estimates across arms.
Results: The 15 arms were from one prospective observational study, five retrospective observational studies, and nine clinical trials. Samples ranged from 9 to 117 patients. All but one study were done at referral centers, and nine specifically excluded patients with elevated serum creatinine at baseline. Mean serum creatinine at entry was 1.2 mg/dl and mean proteinuria was 3.0 g/day. Mean duration of lupus nephritis at entry was 1.1 years. Mean follow-up was 4.5 years. At 1 to 3 years of lupus nephritis, the pooled estimate of ESRD was 4.5% (based on five arms); at 4 to 6 years, the pooled estimate was 6.9% (three arms); at 7 to 9 years, the pooled estimate was also 6.9% (five arms); and at 10 to 11 years, the pooled estimate of ESRD was 3.9% (two arms).
Conclusion: In patients with proliferative lupus nephritis enrolled in studies since 1990, the risk of ESRD over the first decade of lupus nephritis is under 7%. This estimate is largely based on long-term follow-up of clinical trials and studies that excluded patients with renal insufficiency at baseline.

A13

Impact of systemic lupus erythematosus organ damage on unemployment or disability from a population-based cohort

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Background: Systemic lupus erythematosus (SLE) predominantly develops in younger age groups, when many are establishing themselves in the workforce. The development of a chronic, autoimmune condition during this period can have a devastating impact on employment. The objective was to determine the impact of different organ damage in patients with SLE on employment loss from a large, population-based cohort.
Methods: The source of data was from the 2011 to 2012 annual patient-reported survey of the Georgians Organized Against Lupus (GOAL) Study, an ongoing population-based cohort of patients with validated SLE in Atlanta, GA assembled primarily from the Georgia Lupus Registry (GLR). The GLR was supported by the Centers for Disease Control and Prevention and designed to more accurately estimate the incidence and prevalence of SLE. In partnership with the state health department, the GLR was able to access protected health information without patient consent. GOAL Study participants were surveyed regarding employment status at the time of survey completion along with other demographic information. Organ damage was measured using the Brief Index of Lupus Damage. Disease activity was measured using the SLE Activity Questionnaire (SLAQ). Logistic regression analysis was used to measure the association between categories of organ damage and unemployment/disability.
Results: A total of 459 SLE patients were surveyed with a mean age of 46.5 (SD ± 10), 13.4 (SD ± 8.6) years of disease, and 14.2 (SD ± 2.8) years of education; 93.2% were female, 79.5% were black and 14.4% white. One hundred and ninety-seven (42.9%) were working and 262 (57.1%) were unemployed/disabled. The median duration of loss of employment was 7.1 years (IQR 3.1 to 12.3). See Table 1.
Conclusion: In total, 57.1% of SLE patients were unemployed or disabled at the time of the survey. Organ damage from SLE has a profound association with unemployment/disability. In the multivariate model, low education level and depression were independently associated with unemployment/disability. In line with other studies, cardiovascular and renal damage were associated with unemployment/disability. Previous studies have not reported disease activity (SLAQ) as a mediator and

Table 1(abstract A13) Factors related to unemployment/disability in SLE

Sociodemographic factor	Univariate			Multivariate model[a]		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Gender (female vs. male)	0.61	0.28 to 1.34	0.22	0.88	0.25 to 3.03	0.83
Race (white vs. black)	0.30	0.17 to 0.52	<0.001	0.36	0.15 to 0.86	0.021
Education (per year)	0.75	0.69 to 0.81	<0.001	0.75	0.67 to 0.84	<0.001
Depression	4.09	2.68 to 6.25	<0.001	4.52	2.42 to 8.45	<0.001
Currently taking corticosteroids	2.65	1.80 to 3.91	<0.001	2.06	1.18 to 3.62	0.012
Organ damage						
Cardiovascular	7.48	3.49 to 16.03	<0.001	7.78	2.77 to 21.82	<0.001
Renal	5.34	2.04 to 13.97	<0.001	9.38	2.6 to 33.85	0.001
Neuropsychiatric	2.76	1.59 to 4.81	0.001			
Neuropsychiatric, low SLAQ				6.65	1.41 to 31.44	0.017
Neuropsychiatric, high SLAQ				0.93	0.37 to 2.34	0.87

[a]Logistic regression.

should be considered particularly when evaluating neuropsychiatric damage and its association with employment.

A14

Population-based study of preventable infections in hospitalized patients with systemic lupus erythematosus

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Background: Infection is a prominent cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). The proportion of hospitalizations in patients with SLE due to preventable infections is unknown. The objectives of this study were to: determine the proportion of hospitalizations in SLE patients due to preventable infections; and determine whether preventable infections are associated with lengths of stay ≥14 days or mortality.
Methods: This study was a retrospective cohort study using a provincial administrative dataset of hospitalizations (Discharge Abstract Database (DAD)) in Alberta, Canada. A total of 1,626 SLE patients with hospitalizations during fiscal years 2002 to 2009 were identified from the DAD using the International Classification of Diseases-10-CA (ICD-10-CA) code M32.x. Outcome measures of preventable infections (influenza, *Streptococcal pneumonia*, tuberculosis and unspecified pneumonia) were identified during readmissions in 2009/10 using ICD-10 codes. Comparisons between admissions with and without infections were computed using admission and patient characteristics: Student's *t* test and the Wilcoxon rank-sum tests were used for continuous variables and the chi-square test was used for categorical variables. Generalized estimating equation models were constructed to examine associations between infections and the outcomes of long length of stay (dichotomized to ≥14 or <14 days) and mortality.
Results: In total, 312 SLE patients had 504 admissions in 2009/10. Preventable infections accounted for 9.3% of all admissions. Patients with infections had similar demographic characteristics and co-morbidities compared with those without infections with the exception of chronic pulmonary diseases that were more common in those with infections (41.4% vs. 18.3%, $P = 0.003$). Hospitalizations with preventable infections were associated with longer median length of stay (7.5, IQR 4.4 to 23.6 days vs. 4.7, IQR 2.3 to 11.0 days, $P < 0.001$) and higher mortality rates (12.8% vs. 5.5%, $P = 0.047$). Generalized estimated equations revealed that infections were associated with length of stay ≥14 days (OR = 2.59, 95% CI = 1.31 to 5.11, $P = 0.006$) but not mortality (OR = 2.13, 95% CI = 0.81 to 5.62, $P = 0.128$).
Conclusion: Nearly one in 10 admissions in SLE patients previously hospitalized are due to or complicated by preventable infections. The preventable infections are associated with an increased length of stay and therefore greater resource utilization. These results highlight the importance of preventative vaccinations in SLE patients. Further studies are warranted to determine where gaps in vaccination and infection prevention occur in this population.
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A15

Lung cancer in systemic lupus erythematosus

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Background: An increased lung cancer risk in SLE has been suggested in the literature [1]. Our objective was to provide an updated analyses of the lung cancer cases from a multi-site international cohort study, including descriptive statistics of the demographics (age, sex, and race/ethnicity) of cases, as well the of histology.
Methods: Data from an SLE sample of 15,980 SLE patients from 28 centers were analyzed (in Canada, the United States, the United Kingdom, Denmark, Sweden, Scotland, Korea and Iceland). Information on date of birth, sex and race was available, along with the date of SLE diagnosis. Cancer occurrence was ascertained through linkages with regional tumor registries. We assessed the demographic characteristics for all lung cancer cases in the SLE patients, and information on histology types was analyzed from the centers where this information was available.
Results: In the current analyses, 101 lung cancer cases that had occurred after SLE diagnosis were studied. The lung cancer cases were distributed across 21 centers. The average age of the SLE patients at lung cancer diagnosis was 60 years (median 40, standard deviation (SD) 10.9). The average SLE duration at the time of lung cancer diagnosis was 13 years (median 12, SD 10.6). Race/ethnicity was not provided by six centers (35 cases). Of the remaining 66 cases, the majority were Caucasian ($n = 54$, 53.5%) followed by nine African-American, one Asian, one Pacific-Islander and one of unknown racial/ethnic origin. Histological lung cancer type was only provided by 12 centers (59 cases). The most common histological type reported within these 59 cases was squamous cell carcinoma ($n = 15$, 25.4%; 95% CI = 16.1 to 37.8) followed by adenocarcinoma ($n = 13$, 22%; 95% CI = 13.4 to 34.1) and nonsmall-cell carcinoma ($n = 5$, 8.5%; 95% CI = 3.7 to 18.4). The remaining 44% were composed of carcinomas not otherwise specified and a variety of uncommon tumors: large cell, clear cell, solid, bronchoalveolar, adenosquamous, epithelial hemangioendothelioma, oat cell, carcinoid, small cell and mucinous histological types.
Conclusion: In the general population, about 30 to 40% of lung cancer cases are adenocarcinoma, with 20 to 30% squamous cell carcinoma, and 10% large cell carcinoma [2]. Our results suggest a similar distribution, but with a possibly lower proportion of adenocarcinomas, and a higher number of uncommon lung cancer types. Further work is planned to assess other features of these cancers.

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A16

Lymphoma risk in systemic lupus: effects of treatment versus disease activity

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Background: We recently evaluated the risk of malignancy in SLE by linking a multi-site international SLE cohort with regional tumor registries. Across 28 centers, 15,980 patients were observed for 119,846 (average 7.5) person-years. In total, 641 cancers occurred, for an overall standardized incidence ratio (SIR) of 1.14 (95% CI = 1.06 to 1.24). Hematologic malignancies were substantially increased (SIR = 3.01, 95% CI = 2.47 to 3.62), particularly non-Hodgkin's lymphoma (NHL; SIR = 4.36, 95% CI = 3.43 to 5.47) and leukemia (SIR = 1.76, 95% CI = 1.04 to 2.78) [1]. Yet the relative influence of treatment versus disease activity is unknown. Our objective was to determine the relative importance of drugs versus disease activity in mediating the increased risk of lymphoma.

Methods: We performed case-cohort analyses within this multi-site SLE cohort. Adjusted hazard ratios (HRs) for lymphoma were generated in multivariate regression models, for time-dependent exposures to immunomodulators (cyclophosphamide, azathioprine, methotrexate, mycophenolate, antimalarials, glucocorticoids), disease activity (mean adjusted SLEDAI-2K), demographics, calendar year, Sjögren's syndrome, and SLE duration. Partially adjusted models were also performed, using only covariates whose HR CI excluded the null. Sensitivity analyses were performed, lagging cyclophosphamide exposures by 5 years. Medications were treated both categorically (ever/never) and as cumulative doses.

Results: We studied 64 lymphomas (61 NHL, three Hodgkin's) and 4,739 cancer-free controls. As in the general population, lymphoma risk in SLE was higher in males and with age. Lymphomas occurred a mean of 13.1 years (standard deviation 9.8) after SLE diagnosis. Univariate analyses suggested a decreased lymphoma risk within the highest tertile of disease activity (relative to those with the lowest activity) but in fully adjusted models (using all variables listed above), the CI included the null (Table 1). Sensitivity analyses, lagging cyclophosphamide exposures, yielded similar results. In a partially adjusted model (retaining age and highest tertile of disease activity), the HR suggested a twofold lymphoma risk after cyclophosphamide. Despite a trend towards greater cyclophosphamide use in cases versus controls, in fully adjusted models, no drug was estimated to be an independent risk factor. Still, due to correlation, it remains difficult to differentiate the effects of medications from disease activity.

Conclusion: We did not definitively demonstrate an increased risk for any medications, despite a trend to greater cyclophosphamide use in the lymphoma cases. If anything, we noted a protective effect for very high SLE disease activity. Further work will focus on genetic profiles that might interact with medication exposures to influence lymphoma risk in SLE.

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A17

Usual source of care and geographic region are largest predictors of healthcare quality for incident lupus nephritis in US Medicaid recipients

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Background: Little is known about the quality of healthcare delivered to patients with lupus nephritis in the United States and the major determinants of quality remain unknown. We aimed to examine the sociodemographic, geographic, and healthcare system factors associated with performance on a healthcare quality measure in a nationwide cohort of Medicaid recipients with incident lupus nephritis.

Methods: We used US Medicaid analytic extract (MAX) data from 2000 to 2004 containing person-level files on Medicaid eligibility, utilization and payments. We identified patients meeting a validated administrative data definition of incident lupus nephritis, and used this group as the denominator population for the quality metric (QM). The QM numerator assessed receipt of induction therapy with glucocorticoids and another immunosuppressant

Table 1(abstract A16) Results of univariate and multivariate models assessing HR of exposures on lymphoma development in SLE patients

Variable	Univariate HR (95% CI)	Partially adjusted model (95% CI)	Multivarate HR (95% CI)
Cyclophosphamide (CY) ever	1.73 (0.90 to 3.33)	1.99 (1.00 to 3.96)	1.95 (0.61 to 6.22)
Cumulative CY >6 g	1.51 (0.63 to 3.62)	-	0.79 (0.18 to 3.54)
Azathioprine (AZA) ever	0.82 (0.45 to 1.52)	-	1.28 (0.53 to 3.07)
Cumulative AZA >36.5 g	0.49 (0.19 to 1.25)	-	0.46 (0.14 to 1.54)
Methotrexate ever used	0.99 (0.45 to 2.16)	-	0.79 (0.30 to 2.05)
Mycophenolate ever used	1.38 (0.58 to 3.29)	-	1.74 (0.70 to 4.34)
Antimalarials ever used	1.47 (0.80 to 2.69)	-	1.39 (0.69 to 2.78)
Glucocorticosteroids (GC) ever	1.39 (0.75 to 2.56)	-	1.03 (0.40 to 2.64)
Cumulative GC[a] >3.5 g	1.27 (0.75 to 2.17)	-	1.59 (0.69 to 3.67)
Disease activity (second tertile)[b]	1.00 (0.55 to 1.82)	-	1.10(0.56 to 2.14)
Disease activity (third tertile)	0.43 (0.22 to 0.84)	0.49 (0.27 to 0.90)	0.52 (0.24 to 1.12)
Outside North America	1.04 (0.53 to 2.05)	-	1.06 (0.45 to 2.50)
Male	2.47 (1.24 to 4.92)	-	2.21 (1.05 to 4.66)
Age	1.05 (1.03 to 1.07)	1.04 (1.03 to 1.06)	1.05 (1.03 to 1.07)
White race/ethnicity	0.97 (0.55 to 1.71)	-	0.86 (0.46 to 1.59)
Calendar year	1.01 (0.98 to 1.05)	-	0.98 (0.94 to 1.02)
Sjögren's syndrome	1.29 (0.66 to 2.54)	-	1.21 (0.53 to 2.78)

[a]Systemic glucocorticosteroids, considered in prednisone equivalent doses. [b]Mean adjusted SLE Disease Activity-2K (SLEDAI-2K).

(azathioprine, mycophenolate mofetil, mycophenolic acid, cyclophosphamide, cyclosporine A, or tacrolimus) within 90 days of lupus nephritis onset. Patients with end-stage renal disease were excluded. We used multivariable logistic regression models to examine sociodemographic (age, sex, race/ethnicity), geographic (US region), and healthcare (health professional shortage areas, HPSAs, from the Area Resource File) predictors of higher performance. We also examined the restrictiveness of Medicaid benefits in each state, defined by less generous medication coverage policies (mandatory generic substitution, requirements for prior authorization and drug co-payments), and whether the patient's usual source of care was the emergency department or the ambulatory setting (>50% visits).

Results: A total of 974 Medicaid recipients met the definition of incident lupus nephritis. The mean age was 39 years (SD 12), 93% were female, and most were African American (African American 48%, White 27%, Hispanic 13%, Asian 6%). Individuals were geographically dispersed (20% Midwest, 22% Northeast, 34% South, 24% West), and 95% resided in partial or complete HPSAs. One hundred and sixty-four individuals resided in states with more restrictive Medicaid benefits. At 90 days, only 16% of patients met all numerator components of QM1; 45% of individuals received only steroids (mean prednisone dose 28 mg/day), and 3% received an immunosuppressant alone. Among those treated with an immunosuppressant, 31% received azathioprine, 47% received mycophenolate, 14% received cyclophosphamide, and 11% received a calcineurin inhibitor. For 20% (n = 192) of patients, the usual source of care was in the emergency setting. In multivariable logistic regression models, younger individuals were more likely to receive optimal treatment (OR for 18 to 34 years vs. 51 to 64 years = 3.5, CI = 1.6 to 7.6), while those living in the South and Midwest were less likely (OR = 0.49, CI = 0.24 to 0.67 and OR = 0.30 CI = 0.15 to 0.61, respectively). Those whose usual source of care was the emergency department were less likely to receive optimal treatment (OR = 0.47, CI = 0.28 to 0.81). In this adjusted analysis, we did not find significant associations for race/ethnicity, HPSA or Medicaid restrictiveness with QM performance.

Conclusion: Most US Medicaid recipients with incident lupus nephritis in our study did not receive timely induction therapy, and many were treated with high-dose steroids alone. We found significant geographic variation in performance, with the South and Midwest having lower performance than other regions. A substantial number of Medicaid patients with lupus nephritis used the emergency department as a usual source of care and performance on the QM is lower in this setting. These data suggest a need for targeted quality improvement interventions, including increasing access to appropriate ambulatory care for Medicaid recipients with lupus nephritis.

A18

Neuropsychiatric events in SLE: determination of attribution and assessment of outcome

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Background: Neuropsychiatric (NP) events are common in SLE patients and in the general population. Although some events are a direct result of lupus-related autoimmune and inflammatory mechanisms, many are not. The frequency and diversity of NP events in SLE patients pose a challenge in determining their correct attribution and assessing outcome. These are of fundamental importance for the care of individual patients, studies of pathogenesis and the conduct of clinical trials.

Methods: A total of 1,826 patients have been enrolled within 15 months of SLE diagnosis into an international, long-term prospective cohort study. Patients are assessed annually for up to 10 years. At each assessment, all NP events as per the ACR case definitions of 19 NP syndromes are recorded. Attribution of each NP event is determined centrally using two composite attribution rules of different stringency. Additional data collection includes demographic and clinical variables, SLE global disease activity (SLEDAI-2K), SLICC/ACR damage index (SDI), physician assessment of NP events (Likert scale) and self-report mental (MCS) and physical (PCS) component summary scores of the SF-36. Statistical analyses include Cox proportional hazards model, Kaplan-Meier curves, regression and multi-state statistical models.

Results: Upon enrollment into the cohort the proportion of NP events attributed to SLE varied from 19 to 38% and affected 6.1 to 11.7% of patients, depending upon the stringency of the attribution rule. Seizure disorders, cerebrovascular disease, acute confusional states and neuropathies were the most common of the 19 NP syndromes. Changes in SF-36 summary and subscale scores, in particular those related to mental health, were strongly associated with physician assessment of clinical outcomes of NP events. The short-term outcome of NP events was determined by their characteristics and attribution: focal events and those attributed to SLE had better outcomes. Overall, NP events had a negative impact on self-report health-related quality of life (HRQoL) but this was not necessarily true for all types of NP events. For example, most seizures were attributed to SLE, resolved in the absence of anti-seizure medications and were not associated with a persistent negative impact on HRQoL.

Conclusion: The clinical and epidemiological challenge imposed by the high frequency and diversity of NP events in SLE patients can be addressed through the application of composite attribution rules, as well as physician and patient-derived outcome measures.

A19

Seafood consumption and persistent organic pollutants as triggers of autoimmunity among Gullah African Americans

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A19

Background: Local seafood is a dietary staple among the African American Gullah population of South Carolina. High levels of persistent organic pollutants (POPs) have been found in local bottlenose dolphins, sentinel species for human health and consumers of many of the same fish as the Gullah. Links have been established between these bioaccumulating, ubiquitous compounds and deleterious health effects in humans. The objective was to determine whether levels of POPs, specifically per-fluorinated compounds (PFCs), correlate with fish intake and markers of immune dysfunction in genetically at-risk individuals.

Methods: At the onset of the Persistent Organic Pollutants in AutoImmunity (POPAI) study, one-on-one interviews were conducted with Gullah community members to validate a comprehensive environmental exposure questionnaire. The validated questionnaire, including a seafood intake survey, was then administered prospectively to patients with lupus, first-degree relatives of lupus patients, and unrelated nonlupus controls participating in the SLE in Gullah Health (SLEIGH) study. PFC levels (PFOS, PFOA and PFNA), antinuclear antibody titers and other autoantibodies were measured in the serum of participants drawn at the time of their study visit.

Results: Seafood intake questionnaires received from 103 Gullah participants enrolled in the SLEIGH study found 57% consumed locally caught seafood at least once a month and 40% consumed species known to contain high levels of POPs in the Charleston Harbor area. Preliminary results from 33 Gullah controls show that all have measurable serum levels of PFCs (specifically PFOS, PFOA and PFNA) from baseline and follow-up visits 7.3 ± 1.4 years apart, with annual servings of seafood directly correlating with serum PFOS and PFNA ($p = 0.02$ and 0.03). ANA positive controls (48% at baseline) had higher mean levels compared to ANA negative controls for PFOS (75.1 vs 48.2 ng/ml, $p = 0.06$), PFOA (7.0 vs 5.8, p = NS) and PFNA (3.2 vs 2.1, $p = 0.04$).

Conclusion: These ongoing studies address concerns of the Sea Island Gullah community regarding the potential immune health effects of the bioaccumulating pollutants found in local dietary staples such as fish.

A20

Rising prevalence of systemic autoimmune rheumatic disease: increased awareness, increased disease or increased survival?

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A20

Background: The reported prevalence of systemic autoimmune rheumatic disease (SARD) varies markedly worldwide depending on case definitions and population studied. Administrative healthcare databases provide comprehensive longitudinal datasets to estimate changing trends, but their accuracy for identifying SARD must be established, given their use for billing purposes and the diagnostic uncertainty inherent in the disease.

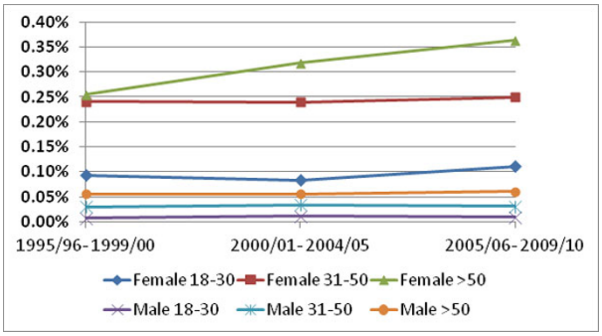

Figure 1(abstract A20) Prevalence of SARD by age and sex.

Methods: In a stable population of over 900,000 adults, we used hospital and physician claims from a large administrative database using two different case definitions for the 5-year period prevalence of SARD from 1995 to 2010. For each 5-year interval, the mid-year population was used as the denominator. For physician claims, we identified SARD using The International Classification of Diseases (ICD)-9 Code 710. For hospital claims we identified SARD using the ICD-9 Code 710 from 1995 to 2004, and the ICD-10 Code M32 from 2004 onwards. Two case definitions were used: ≥5 claims ever for SARD by any physician, or ≥2 SARD claims ≥2 months apart from a specialist within the 5-year period. Prevalence rates by age (18 to 30; 31 to 50; and >50 years) and sex were calculated. The healthcare database was linked with a prescription database, and the proportion of patients treated with glucocorticoids, antimalarials and immunosuppressives was calculated.
Results: The prevalence rate for SARD ranged from 0.08 to 0.013% in 1995 to 2000, and rose to 0.13 to 0.16% in 2005 to 2010, depending on the case definition of SARD. Prevalence rates for females aged >50 rose from 0.25% in 1995 to 2000 to 0.36% in 2005 to 2010, while all other age and sex groups remained stable (Figure 1). The proportion of patients receiving glucocorticoid prescriptions declined slightly from 64% in 1995 to 60% in 2010, while the proportion of antimalarial, moderate and severe immunosuppressive prescriptions increased: 43 to 56%; 24 to 44%; and 3.6 to 6.6% respectively. Rheumatology manpower in the region doubled from 0.47 to 1.2/100,000 from 1995 to 2010.
Conclusion: We found a rise in the prevalence of SARD over the 15-year period. This may reflect improved survival, given the rising rate in females >50, or improved ascertainment due to increasing awareness of SARD among physicians, and increased rheumatology manpower. This study also illustrates changing treatment patterns with less use of glucocorticoids and greater use of antimalarials and immunosuppressives, which may contribute to improved survival. Further attempts at determining diagnostic accuracy and disease outcomes in this population using linkages with clinical databases are underway.

A21

Soluble E-selectin may predict progression of subclinical atherosclerosis, as measured by coronary artery calcium score and aorta calcium score, in women with systemic lupus erythematosus

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Background: Women with systemic lupus erythematosus (SLE) have increased rates of subclinical atherosclerosis and cardiovascular (CV) events [1,2]. Circulating adhesion molecules (CAMs) have been associated with subclinical atherosclerosis in SLE patients [3,4]. We investigated the significance of CAMs in subclinical atherosclerosis progression, as measured by the coronary artery calcium score (CAC) and aorta calcium score (AS) in women with SLE.
Methods: Baseline data collected include demographics and circulating adhesion molecule levels. SLE factors collected included modified SLICC/ACR-DI Damage Index (SDI) (excluding CV outcomes). CAC and AS were measured by electron beam or multidimensional computed tomography at baseline and at one follow-up visit in the Study of Lupus Vascular and Bone Long-Term Endpoints (SOLVABLE). High-risk CAC and AS were defined as CAC >10 and AS >100, respectively. Progression in CAC and AS at follow-up was defined as CAC >10 or AS >100 and >10% increase from baseline. Univariate regression models of CAC and AS with risk factors were examined, and further adjusted for age. CAMs measured were ICAM-1, VCAM-1, soluble E-selectin (sESEL), and CD40L.
Results: Imaging at baseline and follow-up were performed on 142 subjects; baseline AS scans were not performed in 36 subjects (Table 1). Adhesion molecule levels (Table 2) and imaging marker progression (Table 3) results are presented. In age-adjusted models, only sESEL was significantly associated with AS and CAC progression (Table 4).
Conclusion: A higher level of sESEL is associated with progression in AS and CAC in women with SLE. While previous studies have shown CAMs association with subclinical atherosclerosis [3,4], these results suggest sESEL may predict progression of subclinical atherosclerosis, as measured by AS and CAC, in women with SLE.

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Table 1(abstract A21) Baseline demographics

	Age (years)	Disease duration (years)	SLEDAI	SDI	Follow-up time (years)
CAC (*n* = 142)	43.3 ± 9.9	12.0 ± 8.4	3.8 ± 3.5	1.5 ± 1.6	3.25 ± 0.35
AS (*n* = 106)	42.2 ± 9.3	12.0 ± 8.5	4.2 ± 3.6	1.6 ± 1.7	3.26 ± 0.35

Data presented as mean ± SD.

Table 2(abstract A21) Baseline circulating adhesion molecules

	CAC (*n* = 142)	AS (*n* = 106)
ICAM-1	279.9 ± 91.34	276.83 ± 85.79
VCAM-1	978.0 ± 352.15	973.69 ± 360.05
sESEL	62.45 ± 28.70	63.81 ± 26.89
CD40 ligand	5,984.11 ± 2,971.26	6,026.31 ± 2,885.9

Data presented as mean ± SD.

Table 3(abstract A21) Imaging marker progression

	Low risk at baseline		High risk at baseline			
	No progression (%)	With progression (%)	With regression (%)	With progression (%)	No progression (%)	Total number with progression (%)
CAC (n = 142)	103 (72.5)	12 (8.5)	2 (1.4)	21 (14.8)	4 (2.8)	33 (23.2)
AS (n = 106)	67 (63.2)	12 (11.3)	4 (3.8)	23 (21.7)	0	35 (33.0)

Table 4(abstract A21) Adhesion molecules regressed against progression in coronary artery calcium score and aorta calcium score

	CAC progression	AS progression
Risk factor	OR[a] (95% CI)	OR[a] (95% CI)
ICAM-1	0.97 (0.63 to 1.45)	1.30 (0.81 to 2.13)
VCAM-1	1.30 (0.69 to 2.41)	1.85 (0.94 to 3.75)
sESEL	1.68 (1.03 to 2.80)	1.94 (1.08 to 3.60)
CD40 ligand	1.01 (0.65 to 1.56)	0.91 (0.56 to 1.44)

[a]Basis for calculation of OR is 1 SD difference.

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A22

Serum α-chlorofatty acid as a biomarker for baseline subclinical cardiovascular disease in systemic lupus erythematosus

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Objective: α-chlorofatty acid (α-ClFA) is one product of myeloperoxidase activity *in vivo* during atherogenesis [1]. Our study investigates whether serum α-ClFA may be a biomarker for subclinical cardiovascular disease (CVD) in patients with systemic lupus erythematosus (SLE).
Methods: One hundred and eighty-five women with SLE and 186 controls participated in this ancillary study of the Study of Lupus Vascular and Bone Long-term Endpoints (SOLVABLE). Data collection included demographic information, CVD and SLE risk factors, and baseline laboratory assessments. α-ClFA was measured in stored serum by liquid chromatography-electrospray ionization mass spectrometry with selected reaction monitoring detections. Each sample was run in triplicate. Coronary artery calcium (CAC) and aorta calcium (AC) were measured by electron beam computed tomography or multi-detector computed tomography. Calcium scores were calculated using the Agatston method. Outcome measures were the presence of higher risk CAC or AC scores (CAC >10 or AC >100) versus lower risk scores (CAC ≤10 or AC ≤100) [2]. Significant associations were identified with descriptive characteristics, univariate, and multivariate analyses.
Results: Cases had higher baseline levels of α-ClFA than controls (42.2 ± 19.2 fmol/μl vs. 34.5 ± 10.9 fmol/μl, P = 0.014). Cases with lower risk CAC and AC scores had statistically higher levels of α-ClFA compared with controls, while cases and controls with higher risk CAC and AC scores had similar α-ClFA levels (Table 1). In multivariate analyses, SLE had the strongest independent association with higher risk CAC scores, followed by dyslipidemia and age (Table 2). SLE also had the strongest association with higher risk AC scores, followed by history of tobacco use, age, and C-reactive protein level (Table 3). α-ClFA was not independently associated with higher risk CAC or AC scores.
Conclusion: SLE had the strongest independent association with the presence of higher risk subclinical CVD, while serum α-ClFA levels were not independently associated at baseline.
Acknowledgements: This research was supported by R21-HL098907, UL1-RR025741, K24-AR02318, P60-AR30692, P60-AR48098, M01-RR00048, and T32-AR07611 through the National Institutes of Health and the Mary Kirkland Center for Lupus Research and Rheuminations, Inc.

Table 1(abstract A22) Baseline serum α-ClFA levels (fmol/μl) in cases and controls by higher risk versus lower risk CAC and AC scores

Calcium score	Cases	Controls	P value
CAC >10	43.3 ± 21.5	44.0 ± 14.8	0.951
CAC ≤10	42.0 ± 17.6	33.7 ± 10.5	0.010
AC >100	39.3 ± 7.8	37.6 ± 13.1	0.743
AC ≤100	40.4 ± 12.3	33.9 ± 10.5	0.023

Table 2(abstract A22) Multivariate analysis for higher risk CAC scores

Variable	OR	95% CI
SLE	5.81	2.28 to 14.83
Dyslipidemia[a]	5.67	1.50 to 21.36
Age	1.11	1.05 to 1.17
α-ClFA	1.00	0.99 to 1.01

[a]Dyslipidemia defined as total cholesterol >200, low-density lipoprotein >100, high-density lipoprotein <40, triglyceride >150, or lipid-lowering medication use.

Table 3(abstract A22) Multivariate analysis for higher risk AC scores

Variable	OR	95% CI
SLE	3.73	1.59 to 8.78
Tobacco use	2.31	1.13 to 4.74
Age	1.17	1.10 to 1.25
C-reactive protein	1.05	1.01 to 1.11
α-ClFA	1.01	0.99 to 1.02

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A23

Differing environmental risk factors for membranous versus proliferative lupus nephritis

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Objective: Nephritis is a frequent and severe organ involvement in patients with systemic lupus erythematosus. This study examined hormonal and environmental risk factors for biopsy-proven lupus nephritis

(LN), comparing exposures of lupus patients with LN with patients without LN; additional analyses were conducted specifically for the subsets of those with membranous versus proliferative nephritis.
Methods: The Carolina Lupus Study (CLU) is a population-based, case-control study of hormonal, environmental, and genetic risk factors for SLE in the southeastern US (n = 265 incident cases). Diagnosis of LN was determined at the time of enrollment up to 2001 by ACR criteria, with 63 nephritis cases confirmed based on renal biopsy records. An additional 100 biopsy-confirmed LN patients from the CLU study area and time period were identified through disease registries, comprising the nephritis CLU (CLN) cohort. Data collection for both cohorts was a structured 60-minute interview including female reproductive history, lifetime job history, smoking history, use of moonshine and family history of kidney disease, dialysis, hypertension and diabetes. We estimated the association between exposures and risk of developing LN, and LN subtypes (proliferative vs. membranous LN) using logistic regression models, with OR and 95% CI adjusted for age, state, race and education. Analyses were limited to exposures before diagnosis of lupus or LN.
Results: Ninety-seven of the LN cases were classified as proliferative, 39 as membranous and 20 as other classifications (WHO class 2, 6, or unclear from medical record). In 56 patients (36%), diagnosis of LN was concurrent with diagnosis of SLE, in 53 patients (36%) within 2 years of diagnosis and in 47 patients (28%) >2 years after SLE diagnosis. Thirty percent of AA patients versus 10% Caucasians met ACR criteria for LN in the first year following diagnosis. Compared with lupus patients who did not develop nephritis, risk of LN was associated with older age at menarche (P for trend = 0.045), but no other reproductive factors. Reported use of moonshine was significantly higher among LN patients (OR = 3.3, 95% CI = 1.3 to 8.6), and higher among membranous compared with proliferative nephritis (OR = 17.87, 95% CI = 1.55 to 205.89). Likely or possible solvent exposure was associated with a significantly lower risk of nephritis (OR = 0.22, 95% CI = 0.06 to 0.76). There was no association with occupational exposure to silica dust, heavy metals or smoking history after adjusting for covariates. Null associations were seen for most other exposures and risk factors examined. See Table 1.
Conclusion: Our findings provide evidence of an increase risk of LN associated with past use of moonshine, family history of kidney disease, and later age at menarche. Null associations were seen for most other exposures and risk factors examined. Although not previously studied in relation to LN, moonshine use has been associated with chronic kidney disease in other studies as it contains lead and other heavy metals, and is thought to damage the kidney through a variety of mechanisms. The negative association of solvent exposure with membranous nephritis suggests that different pathophysiologic mechanisms may be involved in membranous versus proliferative LN.

Table 1(abstract A23) Reproductive history among LN cases and lupus cases without nephritis stratified by current age (females only)

	Ages 18 to 34 (n = 127)		
Age at menarche	All LN cases (n = 64)	Lupus cases without nephritis (n = 63)	OR (95% CI)
8 to 9	5 (8)	3	1.84 (0.35 to 9.62)
10	3	7 (11)	0.54 (0.11 to 2.65)
11	5 (8)	8 (13)	0.58 (0.15 to 2.29)
12	11 (17)	18 (29)	0.76 (0.27 to 2.18)
13	18 (28)	19 (30)	1.00 (referent)
14	7 (11)	4	1.54 (0.34 to 6.93)
15	9 (14)	2	4.94 (0.88 to 27.92)
16	5 (8)	2	3.57 (0.56 to 22.73)
17 to 18	1	0	-
Trend test (P value)			(0.03)

Missing value (refused) for one lupus nephritis case; three lupus nephritis cases excluded because diagnosis occurred 3 to 4 years before menarche (at age 14); total n = 78.

ADAPTIVE IMMUNITY

A24

B cells at the adaptive-innate immune system interface in SLE

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Background: Accumulating data indicate that inappropriate activation of type I interferon (IFN) plays a key role in the pathogenesis of systemic lupus erythematosus (SLE). Given that IFN can influence B-cell lymphopoiesis in murine bone marrow (BM), we explored the hypothesis that IFN activation in SLE BM has direct effects on B-cell development. Additionally, the impact of B cells on pDC production of IFN was examined.
Methods: Peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) were isolated from 28 patients fulfilling ACR criteria for SLE and from 20 healthy controls. RNA isolates were analyzed for the expression of three to five IFN-regulated genes (IFIT1, IRF7, G1P2, CEB1, C1orf29) by quantitative PCR and IFN-regulated chemokines CXCL10 (IP-10), CCL2 (MCP-1), and CCL19 (MIP-3β) defined in serum and BM supernatant. B-cell subsets were delineated in single-cell suspensions of PBMC and BMMC by multi-parameter flow cytometry. BM and PB pDCs were stimulated with TLR9 (CpG ODN 2006 or 2216) or TLR7 (R848) agonists and intracellular IFN and TNF measured by flow cytometry.
Results: The majority of SLE patients had an IFN signature in the BM (57%), which was even more pronounced than the paired PB. Notably, the early B-cell compartment (consisting of pro B cells, pre B cells, immature B cells and early transitional T1 and T2 B cells) in SLE BM with an IFN signature was associated with a reduction in the fraction of pro/pre B cells, suggesting an inhibition in early B-cell development. However, at the transitional B-cell stage this inhibition was reversed with enhanced selection of B cells into the transitional compartment. The composition of the mature B-cell compartment in IFN-activated SLE BM was notable for an expansion of $CD27^+$, IgD^- switch memory B cells. SLE patients with a BM IFN signature were enriched for a high number of autoantibody specificities (P = 0.003 compared with IFN low), and the degree of IFN activation in the BM correlated with peripheral lymphopenia (P = 0.019) and disease activity (P = 0.05). In order to understand the etiology of IFN activation in SLE BM, we examined the production of IFN by pDCs. CpG induced IFN production in pDCs in a dose-dependent fashion. Notably, a higher proportion of BM pDCs produced IFN compared with paired PB. Moreover, pDCs produced 59% more IFN in the presence of B cells.
Conclusion: This is the first demonstration of an IFN signature in SLE BM. These results suggest that the BM is an important but previously unrecognized target organ in SLE with critical implications for B-cell ontogeny and selection. We postulate that in the setting of IFN activation the stringency of negative selection of autoreactive B cells in the BM may be reduced. Circulating immune complexes and apoptotic fragments in SLE BM may serve as ligands for Toll-like receptors on pDCs contributing to aberrant IFN production and, in turn, B cells may be critical regulators of pDC function.
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A25

In situ cognate interactions between T-follicular helper cells and B cells characterize severe tubulointerstitial inflammation in human lupus nephritis

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Background: In human lupus nephritis, the severity of tubulointerstitial inflammation on biopsy correlates with the risk of subsequent progression

to renal failure [1]. Tubulointerstitial inflammation, and not glomerular inflammation, is associated with *in situ* clonal selection [2] and expansion of B cells reactive with antigens associated with inflammation. Certainly antigen recognition by the B-cell antigen receptor provides signals necessary for B-cell selection. However, additional second signals, derived from antigen-specific T cells or pattern recognition receptors, are required for B-cell proliferation. Therefore, we hypothesized that *in situ* B cells were receiving help from *in situ* T cells.

Methods and results: Consistent with our hypothesis, using four and five color confocal microscopy we observed $CD4^+ICOS^+PD\text{-}1^+$ T cells in close apposition with $CD20^+$ B cells. To quantitate the relationship between these presumptive T_{FH} cells and B cells, we developed novel, automated and therefore unbiased algorithms (using Python/Linux) to define the location of cells expressing multiple surface markers and then to assess the shortest distances between the same or different cell types. These analyses revealed that B cells or T_{FH} cells were never in close approximation with each other. In contrast, an average of 42% of T_{FH} cells in renal biopsies from lupus patients were in very close contact with B cells. Three-dimensional imagining of these conjugates revealed that closely opposed T_{FH} and B cells had formed atypical supramolecular activation complexes containing polarized TCR, LFA-1, MHC class II and ICAM-1 molecules. This suggests that the T_{FH} and B cells in these conjugates are antigenically related. The difference between the observed T:B conjugate rate, and that predicted to arise by chance, was highly significant at up to $P = 10^{-42}$. Finally, qPCR of mRNA captured from *in situ* $ICOS^+$ T cells revealed that they had expression profiles consistent with them being *bona fide* T_{FH} cells.

Conclusion: These data demonstrate that T_{FH} cells are probably contributing to *in situ* humoral immune responses in lupus nephritis. Furthermore, our results indicate that quantitative imaging can be used to reveal important cell:cell interactions in human tissue.

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A26

SLE patients and autoantibody-positive healthy individuals display unique cytokine profiles: shared features of inflammation as well as select features of immunosuppression in autoantibody-positive healthy individuals

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Introduction: Antinuclear antibodies can be detected in up to 25% of the population; however, only 5 to 7% are afflicted with an autoimmune disease. Therefore, many individuals are capable of mitigating the presence of autoantibodies and avoiding clinical disease. The objective of this study was to investigate unique features of SLE patients compared with autoantibody-positive (aAb^+) healthy immune systems and to examine roles that cytokines play in maintaining immune equilibrium in ANA-positive healthy individuals.

Methods: Individuals ($n = 790$) were screened by multiplex, bead-based assays for autoantibodies against: dsDNA, chromatin, ribosomal P, SS-A/Ro, SS-B/La, Sm, Sm/RNP, RNP, SCL-70, Jo-1, and centromere B. Sera from aAb^+ individuals, matched aAb^- controls and SLE patients were tested for 52 cytokines using multiplex bead-based assays and ELISAs. Hierarchical clustering was performed and Kruskal-Wallis tests with Dunn's multiple comparisons were compared including a false discovery rate. Immune cell phenotyping and phospho-flow cytometry was performed on peripheral blood mononuclear cells from aAb^+ and aAb^- healthy individuals. Unpaired *t* tests and Mann-Whitney tests were performed.

Results: Of the screened individuals, 57 individuals were positive for at least one autoantibody (7.2%), with 33.3% being Native American, 57.9% European American, 8.8% African American, and 89.5% female. European American aAb^+ healthy individuals ($n = 31$) and matched aAb^- healthy controls and SLE patients were selected for further analysis. While aAb^+ healthy individuals displayed some similar cytokine patterns to SLE patients, they also displayed a suppressed cytokine profile that included decreased T-cell cytokines (IFNγ ($P < 0.05$), IL-5 ($P < 0.05$), IL-17F ($P < 0.01$)), decreased B-lymphocyte stimulator levels ($P < 0.05$), and increased IL-1 receptor antagonist levels ($P < 0.001$). An increased percentage of B cells was found in aAb^+ healthy individuals compared with aAb^- healthy controls ($P = 0.039$) that consisted of an increased percentage of memory B cells ($P = 0.034$) and a decreased percentage of transitional B cells ($P = 0.028$). Compared with aAb^- healthy controls, B cells from aAb^+ healthy individuals showed significantly increased pSTAT1 and pSTAT3 in response to IFNα stimulation ($P = 0.037$ and $P = 0.040$), increased pSTAT1 in response to IFNγ stimulation ($P = 0.018$), and increased pSTAT3 in response to IL-21 stimulation ($P = 0.041$). $CD4^+$ T cells from aAb^+ healthy individuals showed decreased pSTAT3 in response to IFNγ and IL-2 stimulation ($P = 0.018$ and $P = 0.005$). IFNα, IFNγ and IL-6 stimulation significantly increased pSTAT signaling in monocytes from aAb^+ healthy individuals.

Conclusion: Although aAb^+ healthy individuals exhibit features of inflammation and loss of immune tolerance, they are capable of suppressing these responses by regulatory mechanisms probably no longer functional in patients with autoimmune disease.

A27

Regulatory natural autoantibodies suppress inflammation and SLE disease activity

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Background: Our recent studies have shown that IgM natural antibodies (NAbs) that recognize epitopes on apoptotic cells (ACs) are present from birth and can have potent immunoregulatory properties. These antibodies, by recruiting early complement recognition factors, C1q or mannose-binding lectin, enhance AC clearance by innate immune cells and also mediate suppression of proinflammatory responses induced by Toll-like receptor (TLR) agonists [1,2]. Furthermore, *in vivo* administration blocks the development of inflammatory autoimmune disease in experimental murine models [2]. We therefore explored the mechanistic basis for these properties and assessed for potential clinical relevance.

Methods: Bone-marrow-derived myeloid DCs and macrophages were stimulated with TLR agonists or with immune complexes (ICs) composed of IgG-autoantibody-chromatin or IgG-autoantibody-RNA. We then evaluated production of inflammatory cytokines, and expression of co-stimulatory molecules, which are markers of DC activation. MAPK activation was evaluated by phospho-flow and immunofluorescence microscopy. In a cohort of 120 SLE patients, we examined the relationships between levels of IgM natural autoantibodies to apoptosis-associated antigens, with lupus-associated autoantibodies and features of disease.

Results: In mechanistic studies, during responses to TLR agonists, anti-AC IgM decreased DC activation of the primary MAPKs; ERK1/2, JNK, and particularly p38. This inhibitory response was linked to anti-inflammatory signaling events dependent on nuclear localization of MAPK phosphatase-1, a factor known to also mediate glucocorticoid suppression of immune responses. Anti-AC NAb IgM also suppressed both DNA-IC-induced and RNA-IC-induced cytokine production, and upregulation of CD86 and CD40 on DCs. IgM anti-AC NAb also suppressed IC-mediated MAPK activation [3]. In clinical surveys, IgM autoantibodies to apoptotic cell membranes were commonly detected in healthy humans and SLE patients. In SLE patients, we found that IgM anti-AC levels were significantly higher in patients with low disease activity and less organ damage (by SELENA-SLEDAI, the physician's evaluation and the SLICC damage score). Furthermore, IgM anti-AC levels were also significantly higher in patients without cardiovascular events. In contrast, IgM anti-cardiolipin and IgM anti-dsDNA were significantly higher in patients without renal disease [4].

Conclusion: Our recent studies have demonstrated a direct inhibitory effect of the NAb IgM on inflammatory responses induced by IgG-nucleic acid ICs. Our clinical surveys also contribute to emerging evidence that regulatory anti-AC NAbs may oppose the influence of pathogenic lupus autoantibody-ICs and thereby play roles in maintaining immune homeostasis. These results

support the hypothesis that some IgM autoantibodies are part of a natural immune repertoire that provides protection from certain clinical lupus features.

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A28

Genetic polymorphisms leading to altered T-cell and dendritic cell function cooperate to produce expansion of proinflammatory T-cell subsets in NZB c1 congenic mice

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Background: We have previously shown that B6 mice with an introgressed homozygous NZB chromosome 1 (c1) interval (70 to 100 cM) develop high titers of antinuclear antibodies and severe glomerulonephritis. Using subcongenic mice with shorter c1 intervals, we found that expansion of T_H1, T_H17, and T_{FH} cells was closely associated with the severity of glomerulonephritis. The same expansions were observed using ovalbumin (OVA) as an exogenous antigen, indicating that this was an intrinsic aspect of their immune system. Here we have investigated the role of T cells and dendritic cells (DC) in this expansion.

Methods: OVA-specific T cells from B6 or c1 congenic OT-II TCR transgenic mice were adoptively transferred into B6.Thy1.1 or c1(70-100).Thy1.1 mice. The mice were immunized with OVA emulsified in CFA, sacrificed 2 weeks later, and the proportion of various splenic T-cell subsets determined by flow cytometry, gating on $Thy1.2^+$ (transferred) T cells. Bone marrow-derived DC isolated from 8-week-old c1(70-100), c1(88-100) and c1(96-100) congenic and B6 control mice were cultured in the presence of LPS, imiquimod and CpG, or pulsed with OVA and co-cultured with naïve OT-II T cells. Production of cyto/chemokines (IL-12, IL-23, IL-6) by stimulated DC was analyzed by ELISA or flow cytometry.

Results: Adoptive transfer experiments revealed that the increased IFNγ and IL-17 secreting cell differentiation in c1(70-100) congenic mice arises in part from intrinsic T-cell defects localizing to the NZB c1 96 to 100 and 88 to 96 intervals, respectively. However, OT-II T cells from all mouse strains examined demonstrated enhanced differentiation to T_H1, T_H17, and T_{FH} populations when transferred into c1(70-100).Thy1.1 as compared with B6.Thy1.1 mice. Since DC play an important role in the antigen presentation and cytokine secretion that directs T-cell responses, DC function was contrasted in the various mouse strains. Following TLR stimulation, DC from c1(70-100) mice expressed significantly higher levels of MHC and co-stimulatory molecules, and secreted higher amounts of proinflammatory cytokines such as IL-6 and IL-12. Consistent with altered DC function, OVA pulsed DC from c1(70-100) mice induced significantly increased differentiation of naïve OT-II cells to IFNγ, IL-17 or IL-21 secreting cells as compared with B6 DC.

Conclusion: Our results suggest that a genetic polymorphism in the 70 to 100 interval of NZB c1 congenic mice alters DC function and acts together with intrinsic T-cell defects that map to the 88 to 100 interval to promote the expansion of T_H1, T_H17 and T_{FH} cells in c1(70-100) mice.

A29

Abstract withdrawn

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Abstract withdrawn:

A30

Dysregulation of the serine/threonine phosphatase PP2A contributes to autoimmunity

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Protein phosphatase 2A is a ubiquitously expressed serine/threonine phosphatase that regulates a large number of cellular processes. Levels of the catalytic subunit of PP2A (PP2Ac) are tightly controlled. T cells from patients with systemic lupus erythematosus (SLE) express abnormally high levels of PP2Ac. This is promoted by a lupus-associated SNP and by DNA hypomethylation that alters local transcription factor binding. PP2Ac regulates CREB, Elf-1, and SP1, and could thus contribute to several molecular abnormalities described in SLE T cells. In order to determine whether increased levels of PP2Ac in T cells play a role in the development and/or promotion of autoimmune disease, we genetically engineered a mouse to transgenically express high levels of PP2A in T cells. This isolated abnormality allowed us to evaluate the effects of PP2Ac dysregulation in an otherwise normal immune system.

PP2Ac transgenic (Tg) mice developed modest splenomegaly and lymphadenopathy, but no overt signs of autoimmunity. Susceptibility of PP2Ac Tg mice to immune-mediated disease was evaluated by antibody-induced glomerulonephritis. Glomerular damage and proteinuria were significantly more severe in PP2Ac Tg mice than in nontransgenic littermates. To determine the mechanism underlying increased kidney damage, we analyzed cytokine production by T cells from PP2Ac Tg and control mice. Production of IL-17 was increased ~10-fold in Tg mice. This phenomenon was documented *in vitro* and *in vivo* and was pathologically relevant since blockade of IL-17 abrogated the enhanced susceptibility to glomerulonephritis of Tg mice. These results indicated that increased PP2Ac levels enhance the production of IL-17 in T cells and, through this mechanism, promote immune-mediated organ damage. To determine the molecular pathways through which PP2Ac enhances IL-17 production, we analyzed differentiation of CD4 T cells into effector subsets (that is, Th1, Th2, Th17). Although IL-17A and IL-17F production were constantly higher in PP2Ac Tg T cells, other Th17 characteristics did not differ between Tg and control T cells suggesting that the IL-17-inducing effect occurred independently of Th17 differentiation. Analysis of the *Il17* locus demonstrated that PP2Ac overexpression was associated with local permissive epigenetic changes (increased histone 3 acetylation), which explained enhanced IL-17 production.

We provide evidence that supports dysregulation of PP2Ac in T cells is able to facilitate autoimmune disease by promoting the production of IL-17. This effect is independent of Th17 differentiation and is explained by the capacity of PP2Ac to modify chromatin accessibility at the *Il17* locus by inducing defined epigenetic modifications.

A31

Spontaneous aggregation of the anti-viral MAVS protein in systemic lupus erythematosus: a possible cause of excessive type I interferon production

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Rationale: Patients with systemic lupus erythematosus (SLE) often have evidence of excessive type I interferon production, with increased interferon levels and activation of interferon-inducible genes (interferon signature).

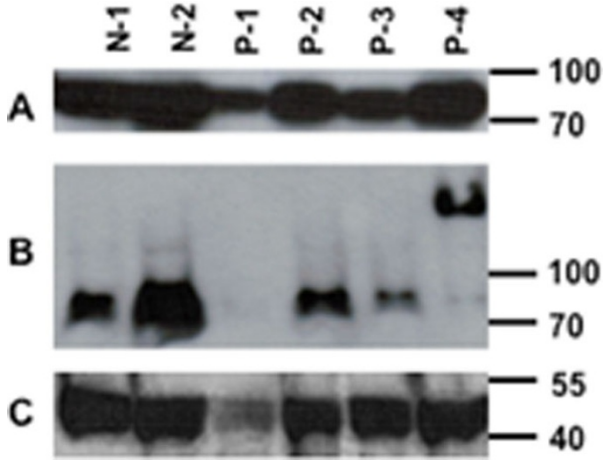

Figure 1(abstract A31)

The mitochondrial adaptor protein MAVS (also known as IPS1, VISA or CARDIF) is a key intermediary in the RIG-I pathway, where viral RNA triggers a conformational change in RIG-I, leading to MAVS activation and activation of IKK and TBK1, with subsequent interferon production driven by IRF-3 and NFκB activation and translocation. A recent report using *in vitro* methods demonstrated that MAVS may form large prion-like aggregates which might stimulate IFN-I activation in a potent and prolonged fashion [1]. We wondered whether such aggregates might be detectable *ex vivo* in SLE patients, and whether they might play a role in the sustained increased production of IFN-I. The aim was to determine whether aggregated MAVS protein is present in blood cells from SLE patients.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from 17 patients fulfilling ACR criteria for SLE, and from nine controls. Thirty million PBMC were lysed and pellets loaded onto semi-denaturing detergent vertical 1.5% agarose gels. After electrophoresis, the proteins were transferred to Immobilon membranes for immunoblotting with anti-MAVS antibody or anti-β-actin.

Results: Six of 17 SLE patients showed clear MAVS aggregation, with essentially all of their MAVS protein in a high-molecular-weight form. None of nine controls had abnormal MAVS. Three of the four SLE patients had nephritis and the fourth had lung involvement. SLEDAI scores of MAVS-aggregate positive SLE patients did not differ from patients with normal molecular weight MAVS. Patient 4 (P-4) shows the aggregated MAVS phenotype in the western blot (Figure 1). MAVS immunoblotting is shown in panel B and actin immunoblotting in panel C. N-1 and N-2 are normal controls. P-1 has less protein loaded and no MAVS band is discernible.

Conclusion: This is the first report of aggregated MAVS in human cells. The relationship of this abnormality to disease needs further investigation, but suggests the possibility that prolonged and increased IFN-I production could result from MAVS aggregation, with the formation of poorly degradable prion-like protein that could signal IFN-I production for prolonged periods.

Reference

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INNATE IMMUNITY

A32

IRF5 expression profiling in SLE patients: new leads to a pathogenic signature?

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Joint linkage and association analysis from multiple laboratories have identified and confirmed polymorphisms in the interferon regulatory factor 5 (*IRF5*) gene that are statistically associated with susceptibility to systemic lupus erythematosus (SLE). *IRF5* SNPs and genetic variants have been consistently replicated and shown to confer risk for or protection from the development of SLE. IRF5 expression is significantly upregulated in SLE patients and upregulation associates with *IRF5*-SLE risk haplotypes. *IRF5* alternative splicing has also been shown to be elevated in SLE patients. Given that human *IRF5* exists as multiple alternatively spliced transcripts, each with potentially distinct function(s), it is important to determine whether the *IRF5* transcript profile expressed in healthy donor immune cells is different from that expressed in SLE patients. Moreover, the exact functional consequence of an *IRF5*-SLE risk haplotype on the profile of *IRF5* transcripts expressed needs to be addressed. Using a combination of standard molecular cloning techniques and recent advances in next-generation sequencing technologies, we examined the profile of *IRF5* transcripts expressed in purified immune cells of healthy donors and SLE patients. By enriching for *IRF5*, we obtained an unprecedented coverage depth >3,000-fold per sample. Molecular cloning was used to generate a full-length *IRF5* variant transcriptome for the analysis of *IRF5* transcript expression in primary immune cells of SLE patients and healthy donors. This method of analysis was compared with other methods that do not rely on the knowledge of an *IRF5* transcriptome; that is, using junction counts and *de novo* junction discovery. Data from these studies support the overall complexity of *IRF5* alternative splicing in SLE and resulted in the identification and isolation of 14 new differentially spliced *IRF5* transcripts. Results from next-generation sequencing correlated with cloning and gave similar abundance rankings in SLE patients, thus supporting the use of this new technology for in-depth single gene transcript profiling. This study provides the first proof that SLE patients express an *IRF5* transcript signature that is distinct from healthy donors, and that an *IRF5*-SLE risk haplotype defines the most abundant *IRF5* transcripts expressed in SLE patients. We posit this newly defined workflow of next-generation sequencing for the rapid enrichment, identification, and quantification of differentially spliced transcripts in donor RNA samples.

A33

Role of MIF gene polymorphisms in systemic lupus erythematosus and prospects for therapeutic intervention

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The cytokine macrophage migration inhibitory factor (MIF) counter-regulates the immunosuppressive action of glucocorticoids and inhibits activation-induced apoptosis. MIF is encoded in a polymorphic locus with a variant allele frequency >5% and high-expression alleles are associated with clinical severity of rheumatoid arthritis, scleroderma, and asthma but improved outcome from pneumonia.

We completed a candidate gene association study in SLE (3195 patients and controls) to examine the relationship between two promoter polymorphisms in *MIF*: a -794 $CATT_{5\text{-}8}$ microsatellite repeat (*rs5844572*), where increased repeat number leads to greater *MIF* expression; and a -173 G/C SNP (*rs755622*) that is in linkage disequilibrium with $CATT_7$. Patients with the high-expression $CATT_7$ allele had lower SLE incidence: OR = 0.63, $P = 0.001$ in Caucasians and OR = 0.46, $P = 0.012$ in African-Americans. Among patients with established SLE, those with serositis, nephritis, and cerebritis had reduced frequencies of low-expression *MIF* genotypes ($CATT_5$) when compared with patients without end-organ involvement ($P = 0.005$ for serositis, $P = 0.023$ for nephritis, and $P = 0.04$ for cerebritis). Plasma MIF levels and TLR4, TLR7, and TLR9 stimulated MIF production reflected the underlying *MIF* genotype of the studied groups. These data suggest that MIF exerts a dual influence on the immunopathogenesis of SLE. High-expression *MIF* genotypes are associated with a reduced susceptibility to SLE, perhaps by contributing to enhanced clearance of infectious agents. Once SLE develops, however, low-expression *MIF* genotypes may protect from ensuing inflammatory end-organ damage [1].

We also examined the therapeutic impact of MIF antagonism in two mouse models of spontaneous SLE. In both the NZB/NZW F1 and the MRL/*lpr* mouse strains, anti-MIF or small molecule MIF receptor antagonism reduced functional and histological indices of glomerulonephritis, MIF-R^+ leukocyte recruitment, and proinflammatory cytokine and chemokine

expression [2]. A humanized anti-MIF developed from our studies has recently entered phase I clinical testing [3].

These data highlight the importance of MIF in the development of autoimmunity and support the potential clinical feasibility of pharmacologic MIF antagonism. We anticipate that MIF antagonists may be most effectively applied in those individuals who, on the basis of their genotype, manifest a MIF-dependent form of autoimmunity.

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A34

Interplay of neutrophils and type I interferons in the development of end-organ damage in SLE

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Patients with systemic lupus erythematosus (SLE) have up to a 50-fold increased risk of developing atherosclerotic cardiovascular disease that cannot be explained by the Framingham risk equation. While both SLE-specific and nonspecific mechanisms have been proposed to play a prominent role in the induction of premature vascular damage in this disease, the exact etiology remains unclear. We have proposed that an imbalance between vascular damage and repair probably induced by IFNα and other type I interferons could play a prominent role in the induction of accelerated atherosclerosis in SLE. Our group and others have recently elucidated the potential role that these cytokines play in the development and progression of premature atherosclerotic disease in SLE and, potentially, in other autoimmune diseases. More recently, a novel role for aberrant neutrophils in the development of autoimmune responses and vascular damage in SLE and other diseases has emerged; in particular, the role that neutrophil extracellular traps may play in the development of this disease and its vascular complications such as endothelial dysfunction and atherothrombosis. The recent description of a distinct subset of proinflammatory neutrophils isolated from lupus patients that induces vascular damage and synthesizes type I interferons has shed new light into a potentially important cell subset implicated in endothelial damage. Finally, novel discoveries pertaining to the interactions of lipoproteins and the immune system may prove quite informative in understanding how premature atherosclerotic plaque develops in lupus.

A35

Metabolic control of systemic lupus erythematosus: convergence of genetic and environmental factors on mitochondrial dysfunction and mTOR reveal treatment targets in lupus

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Systemic lupus erythematosus (SLE) is characterized by the dysfunction of T cells, B cells, and dendritic cells, the release of proinflammatory nuclear materials from necrotic cells and the formation of antinuclear antibodies (ANA) and immune complexes of ANA with DNA, RNA, and nuclear proteins [1]. Oxidative stress and inflammation lead to parenchymal and vascular tissue damage, the latter resulting in accelerated atherosclerosis that is a major cause of mortality in SLE. Activation of the mammalian target of rapamycin (mTOR) has recently emerged as a key factor in abnormal activation of T cells and B cells in SLE [2]. In T cells, increased production of nitric oxide and mitochondrial hyperpolarization (MHP) were identified as metabolic checkpoints upstream of mTOR activation. mTOR controls the expression T-cell receptor-associated signaling proteins CD4 and CD3ζ through increased expression of the endosome recycling regulator Rab5 and HRES-1/Rab4 genes [3], enhances Ca^{2+} fluxing and skews the expression of tyrosine kinases both in T cells and B cells, and blocks the expression of Foxp3 and the generation of regulatory T cells [4]. MHP, increased activity of mTOR, Rab GTPases, and Syk kinases, and enhanced Ca^{2+} flux have emerged as common T-cell and B-cell biomarkers and targets for treatment in SLE [5]. While inactivation and depletion of B cells have shown success in both animal models and patients, blockade of oxidative stress [6], mTOR [7], tyrosine kinases and T-cell-B-cell interaction are also being evaluated as targets for treatment in SLE.

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A36

Microparticles as autoantigens in human and murine lupus

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Systemic lupus erythematosus is a systemic inflammatory disease characterized by anti-DNA production in association with immune complex deposition. These complexes can deposit in the tissue to incite inflammation as well as stimulate cytokine production by plasmacytoid dendritic cells. While the properties of anti-DNA antibodies have been extensively characterized, little is known about the nature of the DNA in the immune complexes although its origin is generally considered to be cell death. Among sources of extracellular DNA, microparticles (MPs) are small membrane-bound vesicles released from activated and dying cells by a blebbing process. As shown by flow cytometry, these particles display DNA as well as other nuclear molecules in an antigenically accessible form as indicated by the binding of monoclonal anti-DNA antibodies as well as plasma from patients with lupus. Furthermore, plasma from patients with lupus contains increased number of particles with bound IgG, suggesting that MPs can form immune complexes found in lupus. To assess whether MPs play a similar role in murine lupus, we used flow cytometry to measure the presence of MPs with bound IgG in the blood of MRL-*lpr/lpr* and NZB/W mice; in addition, we tested the binding of plasma of these mice to MPs from apoptotic cells. The results of these studies showed important differences in the serological findings of the two strains as reflected by the much higher numbers of MPs with bound IgG in the blood of MRL-*lpr/lpr* compared with NZB/W mice. These studies also showed that antibodies from MRL-*lpr/lpr* mice bound much better to MPs from apoptotic cells than those from NZB/W mice. Since particles in NZB/W blood bound to monoclonal anti-nuclear antibodies as well as MRL-*lpr/lpr* plasma, these findings indicate antigenic activity despite the lack of particle IgG complexes in NZB/W blood. Together, these studies indicate important differences in the serological features of the two strains as reflected by the capacity of

antibodies to bind to MPs. These differences may impact on the process of immune complex formation and its consequences as well as the respective role of anti-DNA and other autoantibodies in nephritis in NZB/W mice.

A37

Innate sensors for nucleic acids and lupus pathogenesis

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We continue our efforts to define the pathogenesis of systemic lupus-like autoimmunity in predisposed mouse models by focusing on the role of endosomal nucleic acid-sensing TLRs.

We reported that NZB mice deficient for the common receptor for type I IFNs showed significant reductions in all disease parameters. To differentiate whether the pathogenic effects were mediated by the multiple IFNα subtypes and/or the single IFNβ, we created congenic NZB mice lacking *Ifnb* and found that disease severity was unaltered, strongly implicating IFNα subtypes as the principal effectors. We then documented that long-term treatment of male BXSB mice with an anti-IFNAR antibody of mouse origin reduced serologic, cellular and histologic disease manifestations and extended survival, suggesting that disease acceleration by the *Tlr7* gene duplication in this model is mediated by type I IFN signaling. The efficacy of this treatment was greater when applied relatively early in the disease process, but reductions in some disease characteristics, especially kidney pathology, were evident even when treatment was initiated at later stages, and a transient therapeutic effect was also noted in the MRL-Fas^{lpr} model. The combined findings suggest that antibody-mediated IFNAR blockade may be a useful treatment approach in human SLE and probably other autoimmune syndromes in which these cytokines appear to play a pathogenic role.

We have also hypothesized that engagement of nucleic acid-sensing TLRs may be responsible for spreading the aberrant response beyond ANAs to encompass the broader spectrum of disease-associated autoantibody specificities. Using MRL-Fas^{lpr} mice congenic for the *3d* mutation of the *Unc93b1* gene, in which signaling by all endosomal TLRs (TLR3, TLR7, TLR9) is extinguished, we indeed found reductions not only in autoantibodies against nucleic acids and associated proteins (anti-chromatin, anti-RNP, anti-Sm), but also in a broad panel of autoantibody specificities, particularly against antigens known to contain or bind to nucleic acids, such as cardiolipin, β_2-glycoprotein 1 and myeloperoxidase. Surprisingly, even anti-erythrocyte autoantibodies and hemolytic anemia were significantly reduced in NZB mice congenic for the *3d* mutation compared with wild-type controls. Thus, almost the entire gamut of autoantibodies in lupus can be traced to the initial engagement of nucleic acid-sensing TLRs.

To examine whether engagement of B-cell intrinsic nucleic acid-sensing TLRs is required for autoantibody production *in vivo*, we generated mixed bone marrow chimeras of B6-Fas^{lpr} mice that were either *3d*/IgH^b or WT/IgH^a and measured allotype-specific IgM RF and IgG_{2a} anti-chromatin responses. Strikingly, both autoantibodies were derived almost exclusively from the wild-type donor B cells. This finding underscores the essential significance of B-cell nucleic acid sensors in loss of tolerance and production of autoantibodies in lupus and provides a specific target for intervention.

Evidence suggests that plasmacytoid dendritic cells (pDC), the major producers of type I IFNs, are likely to be involved in the pathogenesis of lupus. One ENU phenovariant identified by Beutler and colleagues, named *feeble*, showed abrogation of both TLR7-induces and TLR9-induced type I IFN and proinflammatory cytokine production by pDCs, while leaving intact pDC development and TLR responses by other cells. The *feeble* phenotype was mapped to a mutation in *Slc15a4*, which encodes the peptide/histidine transporter 1 (PHT1), one of the four members of the solute carrier 15 (Slc15) family of proteins. In preliminary collaborative studies with Beutler and colleagues, lupus-prone B6-Fas^{lpr} mice congenic for the *feeble* mutation showed significant reduction in hypergammaglobulinemia, lymphadenopathy and mortality. These findings provide direct evidence for the role of pDC in the pathogenesis of systemic autoimmunity and suggests that pharmacologic agents that interfere with *Sle15a4* function may be useful in treating lupus and other diseases in which these cells appear to be involved.

A38

Evaluating the B-cell C3d:CR2 innate-adaptive immune interaction as a therapeutic target in lupus

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Background: B-cell targeted therapies are important strategies in human systemic lupus erythematosus (SLE). Previous studies have shown that B-cell complement receptor type 2 (CR2/CD21), along with its C3 activation fragment antigen-bound ligand designated C3d, play essential roles in the innate-adaptive immune interface and development of antibodies to foreign antigens. CR2 acts with CD19 to greatly amplify B-cell receptor signals. We hypothesize that a similar role is played by this receptor-ligand pair in the development of high-affinity IgG autoantibodies in patients with SLE. Prior gene-targeting studies have suggested, however, that CR2 expression may be needed to maintain tolerance. These studies are confounded, however, because not only is CR2 absent but due to murine-specific gene structures another receptor designated CR1 is also deleted at the same time. CR1 is a receptor for complement fragment C4b, whose deficiency in humans and murine models leads to lupus. In addition, a recent report of the first identified human CR2-deficient individual revealed a humoral immunodeficiency and not an autoimmune phenotype. Other recent studies have shown in MRL/*lpr* and (NZB×NZW)F1 mice that the use of soluble CR2 as a potential dominant negative inhibitor led to a substantial decrease in autoantibody titers.

Methods: To address our hypothesis, we developed novel mAbs that disrupt the CR2-C3d interface alone, without affecting the interactions of CR1 with C4b. We immunized $C3^{-/-}$ mice with recombinant human C3d, and $Cr2^{-/-}$ mice with recombinant murine CR2.

Results: The resultant human C3d-reactive mAbs inhibited C3d-CR2 binding, did not recognize intact C3/C3b, and cross-reacted with mouse C3d. Two anti-C3d mAbs, 3d29 and 3d8b, along with control mAb were pre-injected into mice before sheep red blood cell (SRBC) immunization. IgG1 responses to SRBC antigen were substantially decreased, consistent with the interruption *in vivo* of C3d binding to CR2. One resulting anti-CR2 mAb (4B2, IgG_1), which directly blocks binding of C3d to CR2, was injected in wild-type mice and demonstrated no B-cell depletion but maintenance of blockade of CR2 on the B-cell surface for at least 1 month. SRBC immunization of mice pre-injected with mAb 4B2 revealed reduced anti-SRBC levels to levels found in immunized $Cr2^{-/-}$ mice. No anti-idiotype antibodies were detected.

Conclusion: We have developed unique tools to characterize in mouse models of human lupus the pathogenic roles of both the C3d ligand and CR2 components of the CR2-C3d interaction pair.

AUTOANTIBODIES AND TISSUE DAMAGE

A39

PROMISSE: progress in understanding pregnancy complications in patients with SLE

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Pregnancy complications in women with the antiphospholipid syndrome (APS) and/or SLE include recurrent miscarriage, preeclampsia, placental insufficiency, and intrauterine growth restriction (IUGR). The mechanisms leading to placental and fetal injury *in vivo* are incompletely understood and treatment remains sub-optimal. We have identified complement as an early effector in pregnancy loss and/or IUGR associated with placental inflammation in a mouse model of APS and shown that complement activation drives angiogenic imbalance, placental insufficiency and endothelial injury [1-3] (Figure 1). The PROMISSE Study (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus) is a first-time effort to translate

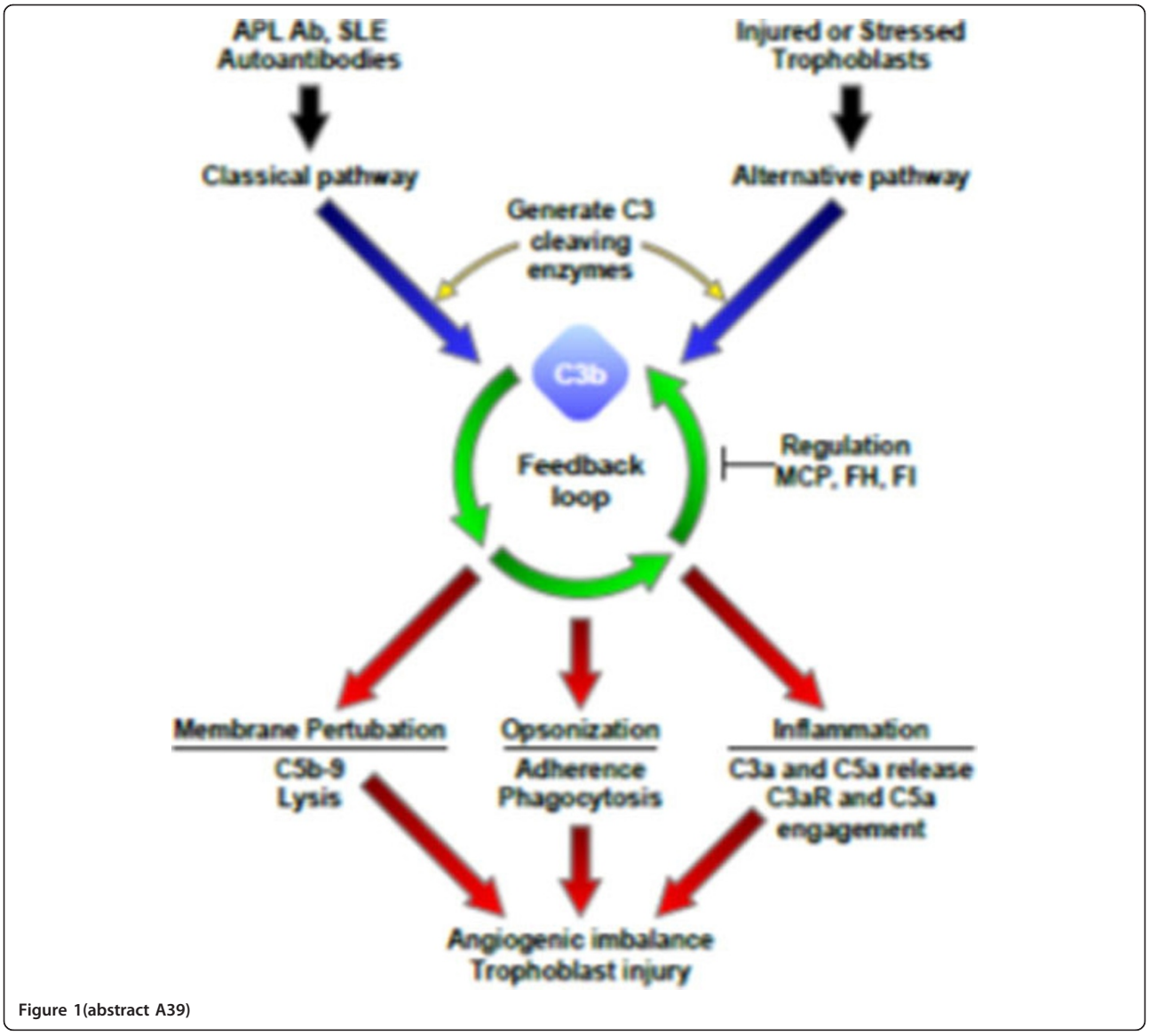

Figure 1(abstract A39)

our novel findings in mice to humans and determine examine the role of complement as a mediator of complications in patients with antiphospholipid (aPL) antibodies and/or SLE. The following discoveries from PROMISSE will be summarized: lupus anticoagulant is the most powerful predictor of poor pregnancy outcomes in aPL-positive patients [4]; activation of complement early in pregnancy can be detected in the blood of women destined to have preeclampsia; circulating anti-angiogenic factors are biomarkers that predict preeclampsia in patients with SLE and/or aPL antibodies and can be released by products of complement activation; and mutations in complement pathway genes that lead to uncontrolled complement activation are associated with preeclampsia in pregnant patients with SLE and/or aPL antibodies [5]. These findings bring us to closer to identifying those at highest risk for pregnancy complications and intervening to block pathways of injury, such as complement.

Acknowledgements: This work is presented on behalf of the PROMISSE Investigators (J Buyon, M Kim, MD Lockshin, CA Laskin, DW Branch, J Merrill, M Petri, L Sammaritano, M Stephenson) and the PROMISSE Collaborators (JP Atkinson, M Triebwasser, SA Karumanchi). This research is supported by grant NIH/NIAMS RO1 AR49772.

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A40

Complement deficiencies and susceptibility to systemic lupus erythematosus revisited

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Recent demonstration of the contribution of more than 30 different SNPs to lupus susceptibility has informed our understanding of pathogenesis of disease. Yet each of these genetic variants is commonly found in the general population and contributes a small effect to lupus susceptibility. In contrast, the almost universal association between C1q deficiency and SLE, as well as high relative risk of other classical components, provides a special opportunity to understand mechanisms of disease.

The complement pathway was implicated in the immunopathogenesis of lupus and other autoimmune disorders decades ago. The apparent paradox that early complement component (C1q, C2 and C4) deficiencies predispose to lupus has been explained by the beneficial roles of these proteins in promoting the clearance of apoptotic cells and immune complexes (ICs). We recently showed that, in the absence of C1q, instead of ICs binding to monocytes, they preferentially engage plasmacytoid dendritic cells (pDC) so providing a powerful stimulus for the production of IFNα, the cytokine with potent immune adjuvant properties [1,2]. We confirmed and extended these findings using microarray analysis of total peripheral blood mononuclear cells and purified monocytes following incubation with SLE ICs in the presence or absence of C1q [3]. We observed that C1q suppressed SLE IC-induced interferon-stimulated genes such as TNFSF13B (BAFF) and TNFSF10 (TRAIL), which are associated with SLE pathogenesis. Interferon-independent pathways that were differentially affected by the presence or absence of C1q in SLE ICs included: multiple cytokines/chemokines (for example, CCL20, CCL23), receptors (for example, CD36, STAB1), and enzymes (for example, RNASE1,2,6, SOD2). Exposure of monocytes to SLE ICs was surprisingly non-inflammatory even when gene expression was examined by microarray.

How then are the lower frequencies of lupus in C4, C2 and C3 deficient patients explained? First, isolated C1q has been shown to exert immunosuppressive properties that have not been identified with C4 or C2. Also, we and others have suggested that C3b may be the key complement protein required for removal of apoptotic cells but that C4b could potentially function in this regard. The lower prevalence of SLE in individuals with a deficiency of C4, C2 or C3 could then be due to only one pathway being defective and, possibly, a functional role for C4b in protection. Future studies will continue to address these questions and guide interventions to promote the safe handling of apoptotic debris and ICs.

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A41

Connecting the molecular dots from inaccessible antigen to organ injury in the pathogenesis of cardiac manifestations of neonatal lupus

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Only 2% of fetuses exposed to anti-SSA/Ro antibodies have cardiac manifestations of neonatal lupus (cardiac NL, referred to herein as congenital heart block), yet these antibodies are present in >85% of mothers whose fetuses are identified with conduction abnormalities in a structurally normal heart. This disparity implies that the antibodies are necessary but insufficient to cause disease, and that the final pathway to fibrosis may be variable: kept totally in check in most fetuses (normal sinus rhythm), subclinical in others (first-degree block) and fully executed in very few (advanced block). A further challenge to defining the pathology of disease is the intracellular location of the target antigens, thus raising questions regarding accessibility to maternal antibodies. Our research has incorporated three components in the presumed cascade from antibody to tissue injury: apoptosis to provide the antigenic complex; macrophage uptake and secretion to drive inflammation and scarring; and persistence of the myofibroblast to drive replacement of healthy cardiac tissue. The totality of experimental data in the last 2 years supports that the watershed may involve conformational properties of Ro60 that influence its surface translocation to an apoptotic cardiocyte surface, its capacity to bind Y RNA and its effect on the biology of the urokinase plasminogen activator (uPA)/urokinase plasminogen activator receptor (uPAR) system. By exploiting murine fibroblast cell lines transfected with Ro60 modified to alter RNA binding sites, very recent data suggest that ssRNA (probably hY3 RNA) is required for surface translocation during apoptosis. However, the accessibility of Ro60 to cognate maternal antibody may be influenced by additional factors. In this regard, Ro60 expressed on the surface of an apoptotic cardiocyte binds domain V of β2GPI with high affinity and effectively blocks binding of anti-Ro60 antibodies. In an important translational step to humans, significantly lower levels of circulating β2GPI are associated with disease as assessed with umbilical cord blood comparing affected and unaffected fetuses. The importance of intact β2GPI as a protective factor became increasingly evident as we continued our search for the molecular explanation of a critical observation that anti-Ro60 antibodies inhibit the uptake of apoptotic cardiocytes by healthy cardiocytes. Although a counter-receptor for Ro60 on the healthy cardiocyte was considered, experiments revealed that anti-Ro60 binding to Ro60 (opsonization, formation of immune complexes) is associated with a conformational change of uPAR resulting in two functional consequences, one of which is a 'don't eat me' signal to healthy cardiocytes. The other is uPA activation leading to proteolytic cleavage of plasminogen and the generation of plasmin. The latter cleaves β2GPI, which renders it incapable of binding to Ro60 thus favoring the continued formation of immune complexes on the apoptotic surface. Moreover, the generation of plasmin also promotes the activation of TGF-β. Accordingly, the uPAR/uPA system plays a profibrotic role in the early and expansive phases of disease and the levels of uPAR, uPA, and plasminogen are increased in affected fetuses compared with healthy siblings. The potential of ssRNA to perpetuate an inflammatory step via a Toll-like receptor (TLR)7 pathway has been experimentally substantiated by the transdifferentiation of human fetal cardiac fibroblasts to a scarring phenotype following exposure to supernatants generated by incubation of macrophages with surrogate immune complexes comprised of Ro60, hY3 and affinity-purified anti-Ro60 antibody. Further evidence for a macrophage contribution to injury was provided by immunohistochemistry, which demonstrated not only a macrophage infiltrate in three hearts from anti-Ro-exposed fetuses dying with cardiac NL, but the formation of multinucleated giant cells. With regard to the fibroblast, we posit that these cells are themselves a major source of TGF-β and that endothelin-1 is one of the components responsible for the profibrosing effects generated by TLR signaling in the macrophages. The potential pathologic role of TLR signaling prompted the initiation of a case-control study in which we demonstrated that the use of hydroxychloroqine, which inhibits endosomal acidification, decreases the risk of cardiac NL. A prospective study to prevent recurrent disease has just been initiated.

A42

MicroRNAs in innate immune responses and autoimmune diseases

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MicroRNAs (miRNAs) are ~22 nucleotide single-stranded RNAs that regulate the stability of target messenger RNAs (mRNAs) via selective binding to specific sites at the 3'-UTR. This triggers repression in translation and mRNA degradation. It has been estimated that ~60% of all mRNAs are under the control of miRNAs. Among the known hundreds of miRNAs, some are considered master regulators controlling either a single or multiple cellular pathways. Some miRNAs are known to affect development and cell differentiation, while others are implicated in immunity and autoimmune diseases. A very interesting example is miR-146a, which has been reported

to be downregulated in systemic lupus erythematosus and upregulated in rheumatoid arthritis. This miRNA plays a dominant role in the regulation of the innate immune responses. The overexpression or underexpression of miRNAs can influence specific targets and pathways, leading to autoimmune disease phenotypes, and this is also supported by some *in vivo* studies. Targeting miRNAs could represent a valid future therapeutic option for autoimmune diseases. This discussion will focus on the current understanding in the function of miR-146a in endotoxin tolerance and cross-tolerance, and how it may contribute to modulate the overproduction of known pathogenic cytokines.

A43

Skin-antigen specific antibodies are detected in UV irradiation and TLR7 agonist induced lupus-like disease in autoimmune prone NOD mice and in pediatric SLE

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Introduction: The role of environmental precipitants in autoimmunity such as systemic lupus erythematosus (SLE) remains unclear. We wished to determine whether UV alone or UV in the presence of TLR7 activation would induce lupus-like disease in an autoimmune mouse model, to explore underlying mechanism(s), and relevance in humans.
Methods: Six-week-old female nonobese diabetic (NOD) mice received repeated weekly 5,000 j/m^2 UVB radiation or 25 μg topical imiquimod or both. Control mice were left untreated. For comparison, nonobese diabetic resistant (NOR) mice were treated with combination therapy (imiquimod + UV). Serum was collected for detection of anti-nuclear antibodies (ANA), desmoglein 3 (Dsg3) antibodies and IFNα by ELISA and detection of proinflammatory cytokines by cytokine bead array. Peripheral blood was collected for cell surface or intracellular staining using flow cytometry. PAS staining of kidney identified the presence of glomerulosclerosis. Dsg3 antibodies were also measured in the serum of children with SLE, type 1 diabetes (T1D) and normal controls.
Results: Imiquimod treatment enhanced ANA and Dsg3 antibody production in NOD mice. Imiquimod + UV induced glomerulosclerosis. Systemic immune activation was detected following combination therapy but not single therapy as evidenced by IL-6, TNFα, IFNγ, and MCP-1. Serum IFNα was significantly elevated in NOD mice following combination therapy. Combination therapy upregulated TLR7 and IFNα expression in the peripheral blood PDCs of NOD but not NOR mice. Anti-Dsg3 antibodies were detected more frequently in children with SLE (5/19) than in children with T1D (1/9) or controls (0/10).
Conclusion: These studies demonstrate that UV light combined with TLR7 engagement induces SLE-like disease in autoimmune prone animals. The presence of anti-Dsg3 antibodies in pediatric SLE suggests that skin-specific autoimmunity occurs in a subset of patients with SLE.

A44

SLE: insights from array-based proteomics

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'Omic'-driven research is exploratory in nature, and seeks to interrogate the entire molecular landscape, with the idea that key pathways or nodes that are aberrant in a disease could be uncovered through a brute-force scan. To date, comprehensive profiling using multiple omics platforms has yielded novel insights on a wide spectrum of diseases, as discussed elsewhere [1].
We recently applied a couple of proteomic and metabolomic approaches to study SLE. In particular, we used planar arrays to uncover novel autoantibodies in SLE, as well as novel serum or urine markers of disease. For the latter, we used planar arrays precoated with antibodies to 274 potential biomarker proteins to interrogate the blood and urine of SLE patients. Thirty of the molecules that were upregulated in SLE sera or urine on these arrays were subsequently validated using independent patient cohorts and orthogonal platforms. In addition to confirming several previously reported increases (including increased serum leptin, osteopontin, OPG, TGF, TNFR-II, and VCAM-1 in SLE), this new study also uncovered several additional proteins to be elevated in SLE.
One example of the novel serum markers being pursued is sTREM-1. sTREM-1 is elevated in the serum of patients with lupus nephritis and within their kidneys. We have also verified that Trem-1 becomes upregulated in mice subjected to anti-GBM nephritis. This molecule may not only be a marker of disease, it may also constitute a novel therapeutic target because Trem-1 blockade curtails nephritis in the anti-GBM experimental nephritis model. Ongoing studies will test the therapeutic potential of this target in spontaneous lupus nephritis. Additional markers uncovered using the arrays will also be discussed.
Omics-based exploratory scans empower us to discern molecules that are differentially expressed in SLE. The challenge ahead is to carefully validate new candidates in order to identify those with the best biomarker or therapeutic potential in SLE.

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A45

Renal flare as a biomarker of incident and progressive chronic kidney disease in patients with lupus nephritis

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Introduction: Flares of lupus nephritis (LN) cause acute kidney injury (AKI), even if serum creatinine (SCr) does not increase. Although classic forms of AKI, such as ischemia, have long been thought to heal without long-term sequelae, it is now clear that AKI events predispose patients to chronic kidney disease (CKD). It was therefore postulated that renal flare (RF) in LN patients promotes CKD.
Methods: To determine whether RF frequency and duration can be used as markers of new CKD or progression of established CKD, we correlated RF with starting and ending SCr levels in the Ohio SLE Study (OSS) cohort.
Results: New-onset CKD occurred in 12/41 patients over a median follow-up of 4.5 years. The CKD group had more RF events than the non-CKD group: 31 (2.59/patient) versus 17 (0.59/patient), respectively, and spent more time in RF (Table 1). Only 8% of the CKD group versus 59% of the non-CKD group had no RF. In OSS patients with established CKD, those who progressed had more RF events than nonprogressors: 13 (1.63/patient) versus 2 (0.29/patient), respectively. In the nonprogressor group 71% had no RF, compared with 37.5% of progressors. Progressors had a significant change in SCr over the study period ($P = 0.0078$). Differences in number of RF and RF duration were not significant between the two groups but tended to be higher in the progressors (Table 2).
Conclusion: In patients with LN, the frequency and duration of RF are biomarkers of new CKD and progression of existing CKD. As new LN therapeutic regimens are developed, targeting RF prevention should be an important goal.

Table 1(abstract A45)

	No CKD ($n = 29$)	New CKD ($n = 12$)	*P* value
Age	31.3	34.3	NS
Male (%)	14	0	-
African American (%)	28	50	-
Mean starting SCr (mg/dl)	0.85 ± 0.15	0.75 ± 0.15	NS
Mean end SCr (mg/dl)	0.83 ± 0.12	1.78 ± 1.95	0.0001
Median time in flare (months)	0	20	0.0003
Mean new RF/year	0.14	0.72	0.0001
Median time in renal health (months)	52	30.4	0.0038

Table 2(abstract A45)

	Nonprogressors (n = 7)	Progressors (n = 8)	P value
Age	39.1	40.5	NS
African American (%)	14	62.5	-
Mean starting SCr (mg/dl)	1.94 ± 0.83	2.15 ± 0.81	NS
Mean end SCr (mg/dl)	1.56 ± 0.87	4.0 ± 2.45	0.0093
Mean new RF per year	0.10	0.41	NS
Median time in renal health (months)	48	25	0.0560

A46

Cytokine profiles of lupus patients with or without nephritis

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Background: It remains unclear why some patients with SLE develop lupus nephritis (LN) and others do not. A reasonable hypothesis is that the inflammatory/immunomodulatory mechanisms in patients with LN are either qualitatively different than in nonrenal (NR) lupus, or that similar pathways are activated but the magnitude of activation is greater in LN. Studies of individual or small groups of candidate cytokines support this idea. To test this hypothesis we examined baseline serum and peripheral blood mononuclear cell (PBMC) cytokine profiles of SLE patients with active LN or NR (mainly arthritis) lupus before entry into a clinical trial of Laquinimod (Teva Pharmaceuticals) as an adjunct anti-inflammatory agent for SLE. We sought to determine whether NR or LN was associated with a distinct pattern or level of cytokine expression.
Methods: Serum and PBMC from 49 NR and 16 LN patients were collected. PBMC were cultured in medium with or without lipopolysaccharide (LPS), phytohemagglutinin (PHA), or tetanus toxoid (TT). PBMC proliferation was measured after 48 hours, and 38 cytokines were measured in the PBMC-conditioned supernatant by Luminex multiplexing. The same cytokines were measured in the sera.
Results: LN patients were younger (37 ± 9 vs. 47 ± 14, $P < 0.006$), had a higher serum creatinine (91.6 ± 28.6 vs. 69.6 ± 16.5 μmol/l, $P < 0.0003$), higher BILAG score (13.6 ± 8.2 vs. 7.2 ± 3.2, $P < 0.0001$), higher baseline corticosteroid dose (18.3 ± 1.3.3 vs. 8 ± 8 mg/day, $P < 0.004$), and were more likely to enter the study taking a concomitant immunosuppressive agent (MMF; 50 vs. 22%). There were no quantitative differences in NR and LN serum cytokine levels for most analytes that were within the limits of detection. Eotaxin and soluble TNF receptor II were greater in LN sera ($P = 0.037$ and $P = 0.022$, respectively). Interestingly, IL-17 was not detectable in any LN sample, but was present in 17% of NR sera. LN and NR PBMC proliferated equally well to LPS and PHA, but in response to TT proliferation of NR PBMC was greater than LN PBMC ($P < 0.0001$). TT did not induce LN PBMC cytokine production. LPS and PHA induced significant increases in production of 24 of the 38 measured cytokines from PBMC, but there were few differences between LN and NR. LPS-induced IL-7 and IL-12 levels were higher from LN PBMC ($P < 0.02$), and PHA induced more IL-5 production by NR PBMC ($P = 0.008$).
Conclusion: Unexpectedly, no cytokine signature that distinctly separates LN from NR emerged from this survey. This may be due to the greater baseline level of immunosuppression in LN patients, but even with immunosuppression the LN was very active. It is intriguing to speculate that differences, perhaps genetic, in the kidney's response to inflammation, rather than differences in circulating proinflammatory cytokines, determine susceptibility to LN in SLE patients.

A47

Which lupus trial endpoints best reflect clinical judgment or biomarker improvement?

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Background: Outcome measures used in clinical trials of lupus are complex and difficult to interpret. With a plethora of new treatments in development and no objective gold standard to define efficacy, a better understanding of what the different endpoints signify would be helpful in designing more efficient trials and in informing clinicians in practice who need to interpret the results.
Methods: Ninety-one patients from the Oklahoma Lupus Cohort (five males, mean age 41) were identified with two visits at which SLEDAI and BILAG scoring had been performed and active disease (SLEDAI >6) was present at the first visit. Each was evaluated by physician judgment as the same, improved or worse at the second visit based on clinical records. Serum cytokine levels were measured by xmap multiplex bead-based assay.
Results: At baseline, the mean (SD) PGA, SLEDAI and BILAG scores were 1.75 (0.37), 10.0 (4.09) and 15.1 (6.54). Sixty-eight patients were ranked as improved, 23 as the same or worse at the follow-up visit. The SLE Responder Index (SRI) and BILAG-based Composite Lupus Assessment (BICLA) were compared. Endpoints using these constructs restrict medication use. SRI and BICLA without medication criteria captured physician-ranked improvement (PRI) with 85.3% versus 76.5% sensitivity and 73.9% versus 78.3% specificity. With medication limits, fewer patients were responders, but specificity increased to 82.6 and 95.6%. Similar trends were observed for modified SRI scores (SRI3 and SRI5). Spearman rank correlations to PRI were: SRI3 = 0.605, SRI4 = 0.563, SRI5 = 0.541, BICLA = 0.492 (all $P < 0.000001$). All nine patients who improved by BICLA but not SRI failed to achieve four-point improvement in the SLEDAI, which requires complete resolution of one or more disease features. However, seven were rated as significantly improved by PRI. All 15 responders to SRI and not BICLA failed to improve in every organ, but 12 were rated as improving by PRI. Biomarkers could provide an objective standard to compare clinical measures. Exploratory evaluation of serum cytokines might allow some preliminary modeling. In the current study IL-6 was only detectable in a minority of patients (n = 29) but decreased significantly in those patients who improved: PRI $P < 0.001$, SRI3 and SRI 4 $P = 0.003$, SRI5 $P = 0.001$, BICLA $P = 0.005$.
Conclusion: SRI5 and BICLA, with the addition of medication restrictions, may be the most specific measures for improvement despite risking loss of sensitivity, and could provide the most meaningful proof of efficacy in an appropriately powered clinical trial. Shortfalls of SRI and BICLA are usually due to the BICLA requiring only partial improvement but in all organs versus SRI requiring full improvement but not necessarily in all organs. Physician's overall opinion corresponds as well as or better than formalized endpoints to improvements of IL-6 in an exploratory biomarker analysis of a lupus patient subset.

A48

'MiLES' population-based survey of the incidence and prevalence of systemic lupus erythematosus in Southeastern Michigan

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Background: We estimated the incidence and prevalence of systemic lupus erythematosus (SLE) in a sociodemographically diverse southeastern Michigan source population of 2.4 million.
Methods: SLE cases fulfilling American College of Rheumatology (ACR)SLE classification criteria (primary case definition) or rheumatologist-judged SLE (secondary definition) and residing in Wayne or Washtenaw Counties during 2002 to 2004 were included. Case finding was performed from six source types, including hospitals and private specialists. Age-standardized rates were computed and capture-recapture performed to estimate under-ascertainment of cases.
Results: Overall age-adjusted SLE incidence and prevalence per 100,000 were 5.5 (95% CI = 5.0 to 6.1) and 72.4 (95% CI = 70.4 to 74.4); capture-recapture adjusted estimates were 5.6 (95% CI = 5.1 to 6.2) and 71.8 (95% CI = 69.8 to 73.8). For all women the incidence was 9.3/100,000; prevalence was 128/100,000. SLE prevalence was 2.4-fold higher in blacks than whites, and 10-fold higher in women than men. Among the incident cases (ACR definition), mean age (± SD) at diagnosis overall was 39.2 ± 16.6 years. Blacks had a higher proportion of renal disease and end-stage

renal disease (40.3% and 15.1%) versus whites (18.7% and 4.5%); blacks with renal disease were diagnosed with SLE at significantly younger age (33.9 ± 15.0 vs. whites 41.9 ± 21.3, $P = 0.04$).

A49

Sociodemographic characteristics of SLE patients in a large metropolitan area with a high Afro-Caribbean population

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Background: Systemic lupus erythematosus (SLE) is characterized by a wide spectrum of manifestations and severity, frequently affecting women and ethnic minorities. Health disparities among ethnic groups adversely impact medical care access, treatment choices and long-term outcomes. Over the past 30 years our lupus cohort has changed to include many patients of Afro-Caribbean ethnicity. Understanding ethnic and sociodemographic characteristics is critical to designing strategies to decrease health disparities and improve patient care.
Methods: A demographic questionnaire was distributed to lupus patients from June 2009 to May 2012. All patients met ≥4 1982 ACR SLE criteria. Questions concerned date of diagnosis, place of birth, ethnicity, religion, main language, marital status, housing, education, employment, financial support, disability and medical insurance. Descriptive statistics were compared with similar demographic studies done in 1980 and 1994, including 164 and 169 patients respectively.
Results: One hundred and fifty patients participated in the survey; 90% were women with average disease duration of 15 (0.4 to 52) years. Forty-seven percent were born in North America, 37% in the Caribbean and 15% in other regions, with a similar distribution in 1994 (47% North America and 45% Caribbean), but a notable change from 1980s demographics, with 74% born in North America and 22% in the Caribbean. The most common ethnicities were Black non-Hispanic (68%) followed by Black-Hispanic (17%) and White-Hispanic (5%). Currently the main spoken language is English (93.3%). Only 21.3% are married, with 54% never married. Eighty percent completed a high-school education, and 26.2% have full-time jobs while 51% are unemployed. The most common occupations are childcare, healthcare, and clerical. Average adjusted gross income is below New York State income ($39.438 per year vs. $59,519 per year). A total of 47.3% of patients receive disability with 40% reporting disability as the main source of financial support, an increase in disability status compared with 1980 (38.4%) and 1994 (46.1%). Access to healthcare and insurance plans increased compared with 1980 (53% Medicaid and 20.1% Medicare vs. 46% self-pay, 36% Medicaid and 20% private). Drug plan coverage is available for 85% of the current cohort.
Conclusion: Although genetic variations highly influence disease patterns, adverse sociodemographic factors negatively influence disease course. Most of our patients come from Caribbean communities with limited education, high unemployment rate, low income and high disability rates. Despite improved access to healthcare, sociocultural barriers may limit access to optimal medical therapies, necessitating interventions to decrease health disparities. Studies comparing clinical characteristics and their influence on outcome of Afro-Caribbean populations and other ethnicities will help to improve medical care for this population.

A50

High score on PREDICTS is associated with 10-fold increased odds for the progression of subclinical atherosclerosis in SLE

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Background: Increased oxidative stress is a major contributor to atherosclerosis (ATH). Patients with SLE demonstrate high oxidative stress and increased ATH. Our group and others have reported several biomarkers and demographic variables associated with increased oxidative stress, including proinflammatory HDL (piHDL), elevated leptin, homocysteine, and increased age - each associated with subclinical ATH in SLE. Can these biomarkers of oxidative stress be combined into a risk profile that better predicts progression of atherosclerosis?
Methods: Female SLE subjects not taking statins had B-mode and Doppler scanning of carotid arteries at baseline and 18 to 36 months later. Many biomarkers were tested and those separating plaque presence were chosen for additional analysis. Antioxidant function of HDL was measured as the change in fluorescence intensity caused by oxidation of DCFH after test HDL was added to standardized normal LDL. Values >1.0 indicated piHDL. Plasma levels of leptin and sTWEAK were measured by ELISA; homocysteine was determined by HPLC.
Results: Follow-up ultrasounds were completed on 210 SLE women. Overall, 21% (38) of SLE patients had new or larger plaques. Factors associated with plaque progression on bivariate analysis included the baseline presence of plaque ($P < 0.001$), increased age ($P < 0.001$), piHDL ($P = 0.003$), high leptin levels ($P = 0.004$), high sTWEAK levels ($P = 0.004$), and diabetes ($P = 0.003$). Although piHDL was the strongest predictor for plaque progression on multivariate analysis (OR = 5.8, 95% CI = 2.1 to 16.7), with a negative predictive value of 89%, the positive predictive value was only 46%. We used a random forests model to determine which variables were most predictive of plaque progression, and the most significant cutpoints to dichotomize each variable. The most significant predictors were age >48, piHDL, high leptin values ≥34 ng/dl, high sTWEAK >373 pg/ml and high homocysteine (≥12). We then created a PREDICTS cardiovascular risk variable, with low oxidative stress risk defined as zero to two predictors, and high stress defined as ≥3 predictors or one predictor plus diabetes. The high PREDICTS variable had a negative predictive value for plaque progression of 88%, but the positive predictive value was 63%. In multivariate analysis controlling for other cardiac risk factors and disease factors, patients with high PREDICTS had a 10.2-fold increased odds for plaque progression (95% CI = 3.9 to 27.0), and 2.1-fold increased odds for the highest quartile of IMT progression/year (95% CI = 1.05 to 4.4).
Conclusion: Formation of a cardiovascular risk model that incorporates several biomarkers and age may provide a more complete means to identify SLE patients at risk for progression of atherosclerosis.
Acknowledgements: Body of work supported by NIH (MM), LRI (BH), ALR (BH), and ACR (MM).

A51

Thinking toward improved treatments of systemic lupus erythematosus

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In 2012, recommended therapies for SLE include antimalarials, glucocorticoids, azathioprine, mycophenolate mofetil (or myfortic acid), cyclophosphamide, and other immunosuppressants. Belimumab has been added recently. Most are targeted toward adaptive immune responses. We now suspect that several pathways in innate immunity are also critical to driving SLE, including dendritic cells (major source of IFNα), and monocyte/macrophages that appear central in the damage that occurs to renal tissue in lupus nephritis. Abnormalities in neutrophils may also drive IFNα production and damage to endothelial cells. Therefore, treatments that modify these innate immune cells are of great interest. Antimalarials primarily suppress antigen-presenting cells (APC), including TLR activation; clinically they suppress disease activity and damage, but not strongly. Glucocorticoids suppress DC, monocytes and lymphocytes, with reduction of trafficking of proinflammatory cells to target tissues, but they are quite toxic. Belimumab is directed primarily at prevention of B-cell maturation and has clinical benefits that are not large when added to standard therapies.
Among the potential new therapies that influence APC is Laquinimod, a quinoline derivative administered orally, which has recently been shown to reduce the number of new MRI lesions and disability in patients with multiple sclerosis. A recent study in murine EAE shows that Laquinimod suppresses activity of DC, and prevents monocyte/macrophages from accessing the CNS. The suppressed APC functions result in reduced number of effector T cells (Th1, Th17) in target tissue. In addition, Laquinimod induces regulatory T cells and myeloid regulatory $CD16^+LyC6^+$ cells that on adoptive transfer suppress clinical disease. We have recent

data, submitted for the 2012 ACR meeting, showing similar immune alterations in a murine model of lupus nephritis, including dramatic benefits on protection of young mice from clinical disease, and regression of disease in mice started on treatment after developing heavy proteinuria. Renal damage is minimal.
Downregulation of innate immune cells to minimize their activation of adaptive immunity, and their ability to invade and initiate damage target tissues should result in not only less active acute disease but also less future damage. This approach might be discussed at the Whistler meeting.
Acknowledgements: The Laqinimod experiments in murine lupus were supported by Teva Pharmaceuticals, Ltd.

A52

Hopkins Lupus Cohort: assessment of treatment effects

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Introduction: The Hopkins Lupus Cohort is a longitudinal study in which all SLE patients are seen quarterly, by protocol, by one rheumatologist. The specific aims include prevention of organ damage. In this abstract, two projects - noncalcified plaque (NCP) and treatment of antiphospholipid antibodies - will be reviewed.
Methods: For the study on NCP, 64-slice (n = 106) or 320-slice (n = 121) coronary multidetector computed tomography (MDCT) was performed in 227 patients with SLE. The MDCT scans were evaluated quantitatively by a radiologist, using dedicated software. The NCP score was a sum of plaque severity multiplied by the plaque composition divided by the number of vessels examined.
For the study on treatment of antiphospholipid antibodies, we studied 1,795 SLE patients (56% Caucasian, 37% African American, 93.3% female, mean age 37.0 ± 12.5) with no previous thrombosis prior to entry in the cohort. The primary outcome was first thrombotic event (arterial or venous). Univariate analysis and multivariable modeling were used to examine associations between prednisone, hydroxycholoquine, and NSAID use with the risk of thrombosis.
Results: The multiple regression model for the mean level of NCP is shown in Table 1. The multiple regression model for prevention of thrombosis is presented in Table 2.
Conclusion: For NCP, methotrexate increased NCP, possibly via homocysteine. For thrombosis, hydroxychloroquine significantly reduced thrombosis, but prednisone increased it. SLE longitudinal cohorts can address many clinical questions that are not suitable for clinical trials.

Table 1(abstract A52)

Variable	Effect on mean NCP score (95% CI)	*P* value
Age (per 10 years)	0.085 (0.057 to 0.113)	<0.0001
Low BMI (vs. normal)	-0.096 (-0.175 to -0.018)	0.017
High BMI (vs. normal)	0.002 (-0.080 to 0.084)	0.96
Hypertension	0.065 (-0.005 to 0.136)	0.068
History of low C3	0.090 (0.020 to 0.161)	0.012
History of ant-dsDNA	0.031 (-0.042 to 0.103)	0.40
Male sex	0.087 (-0.017 to 0.191)	0.10
Methotrexate current	0.279 (0.113 to 0.445)	0.0011

Table 2(abstract A52)

	Subgroup	Thrombotic events	Rate of events/1,000 person-years	Rate ratios (95% CI)	*P* value
Current prednisone	None	52	10.6	1.0 (Ref. Gp)	
	1 to 9 mg/day	40	16.4	1.6 (1.1 to 2.4)	0.025
	10 to 19 mg/day	49	34.3	3.3 (2.2 to 4.8)	<0.0001
	20+ mg/day	43	71.8	6.5(4.3 to 9.8)	<0.0001
Cumulative prednisone dose	None	29	13.1	1.0 (Ref. Gp)	
	<1 year (10 mg/day)	37	23.8	1.6 (1.0 to 2.6)	0.075
	1 to 3 years (10 mg/day)	30	19.0	1.8 (1.1 to 3.1)	0.026
	3 to 10 years (10 mg/day)	45	25.5	3.0 (1.8 to 5.2)	<0.0001
	>10 years (10 mg/day)	9	24.3	3.7 (1.5 to 9.4)	0.0056
Current HCQ	No	95	29.0	1.0 (Ref. Gp)	
	Yes	90	14.6	0.5 (0.4 to 0.7)	<0.0001
HCQ use	Never	57	32.2	1.0 (Ref. Gp)	
	Past (not current)	27	27.6	0.9 (0.6 to 1.5)	0.81
	<6 consecutive months	16	20.0	0.6 (0.3 to 1.0)	0.056
	>6 consecutive months	64	13.8	0.5 (0.3 to 0.7)	0.0003
NSAID use	No	146	21.6	1.0 (Ref. Gp)	
	Yes	37	13.9	0.6 (0.4 to 0.9)	0.017
Aspirin use	No	137	17.9	1.0 (Ref. Gp)	
	Yes	49	28.0	1.6 (1.2 to 2.3)	0.0038

A53

Risk of pulmonary embolism and deep vein thrombosis in systemic lupus erythematosus: a population-based cohort study

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Background: A recent hospital-based study suggested a 10-fold increased risk of pulmonary embolism among individuals with systemic lupus erythematosus (SLE) in the year following hospital admission. It is unknown whether the risk is similar among the nonhospitalized SLE population. We estimated the risk of incident pulmonary embolism (PE) and deep venous thrombosis (DVT) events, as well as the associated time trend, among incident cases of SLE compared with general population controls using physician billing and hospitalization data for the entire province of British Columbia, Canada (~5 million).

Methods: Our data included all visits to health professionals and hospital admissions covered under the province's universal healthcare plan from 1 January 1990 until 31 December 2007 for all individuals ≥18 years of age. We conducted a matched cohort study among patients meeting the following criteria: ≥18 years of age, and new diagnosis of SLE based on the following algorithm: one ICD code for SLE on rheumatologist visit billing data or on hospitalization data, or two ICD codes for SLE at least 2 months and no more than 2 years apart on a physician visit by a nonrheumatologist. Controls were selected from the general population, on a 10:1 ratio for each case, matched by birth year, sex and calendar year of exposure. The outcomes, PE and DVT, we identified based on one ICD code for PE in hospitalization data; and one ICD code for DVT in either outpatient or hospitalization data. We estimated relative risks (RRs) of PE and DVT in SLE cases compared with matched general population controls, after adjusting for age, sex, comorbidities, trauma, fracture, surgery, and hospitalizations.

Results: Among 5,156 individuals with SLE, 54 developed PE and 92 developed DVT. Compared with age-matched, sex-matched, and entry-time-matched controls (n = 51,560), the RRs were 4.9 (95% CI = 3.4 to 6.8) for PE and 4.5 (95% CI = 3.5 to 5.7) for DVT. These RRs attenuated slightly after adjusting for covariates, but remained significant (Table 1). When we evaluated the impact of follow-up time, the RRs for PE, DVT and PE or DVT in SLE patients as compared with non-SLE cases were the largest in the first year (Table 2). The estimates decreased over time and were not significant after 5 years of follow-up with the exception of DVT.

Conclusion: This is the first population-based study assessing the risk of PE and DVT in patients with SLE. These findings support increased monitoring of venous thromboembolic complications and risk factors in SLE patients, especially during the first year after disease onset.

Table 1(abstract A53)

	SLE	Non-SLE
PE		
Cases (*n*)	54	114
Incidence rate/1,000 person-years	2.5	0.5
Age-, sex-, entry-time-matched RR (95% CI)	4.9 (3.4 to 6.8)	1.0
Multivariable RR (95% CI)	4.6 (3.3 to 6.4)	1.0
DVT		
Cases (*n*)	92	214
Incidence rate/1,000 person-years	4.3	1.0
Age-, sex-, entry-time-matched RR (95% CI)	4.5 (3.to 5.7)	1.0
Multivariable RR (95% CI)	4.1 (3.2 to 5.2)	1.0
PE or DVT		
Cases (*n*)	131	295
Incidence rate/1,000 person-years	6.2	1.3
Age-, sex-, entry-time-matched RR (95% CI)	4.7 (3.8 to 5.7)	1.0
Multivariable RR (95% CI)	4.3 (3.5 to 5.3)	1.0

Relative risk of incident PE and DVT according to SLE status.

Table 2(abstract A53) Incidence rates and relative risks for PE, DVT and PE or DVT in patients with SLE during follow-up

	<1 year	1 to 5 years	>5 years of follow-up
PE events	31	20	3
DVT events	45	36	11
PE or DVT events	70	49	12
Incidence rate of PE/1,000 person-years	6.7	1.6	0.6
Incidence rate of DVT/1,000 person-years	9.8	3.0	2.3
Incidence rate of PE or DVT/1,000 person-years	15.5	4.1	2.6
Age-, sex-, entry-time-matched RR of PE (95% CI)	18.4 (9.9 to 35.5)	3.2 (1.8 to 5.3)	1.0 (0.2 to 3.1)
Age-, sex-, entry-time-matched RR of DVT (95% CI)	10.2 (6.6 to 15.8)	3.0 (2.0 to 4.4)	2.5 (1.2 to 5.0)
Age-, sex-, entry-time-matched RR of PE or DVT (95% CI)	13.6 (9.4 to 19.8)	3.0 (2.2 to 4.2)	1.7 (0.9 to 3.2)

OTHER

A54

Poor methodological reporting in lupus clinical trials found in Cochrane reviews

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Background: Results of randomized clinical trials depend on the credibility of the methods reporting to support study findings.
Methods: We studied 24 trials from those in the Cochrane Database of Systematic Reviews with 'lupus' in the title and were printable. Each paper was scored by one reviewer using methodological criteria for design, allocation [1], blinding [2-4], reporting and imputation [5]. Scores used yes, no, or ? when it was unclear. Yes *n* (integer %) for all 24 papers are reported for each criterion.
Results: Design: Four (17%) papers had a sample size justification; 22 (92%) contained two groups and two (8%) contained three groups. Five (21%) stratified patients; yet two (8%) used stratification in the analysis.
Allocation: Six (25%) stated random numbers generated and three (12%) blocked the balance associated with the allocation ratio; yet zero (0%) used blocking in the analysis. Six (25%) used a randomization list concealed from the person deciding patient eligibility, zero (0%) provided an audit trail for randomization, one (4%) stated randomization integrity. Seven (29%) mentioned the randomization constructed with a computer program or random number table.
Blinding: Four (17%) stated the person deciding on the patient eligibility was blinded to block structure and eight (33%) claimed the study was double blinded, even though it was not clear who the two were; indeed one was really triple blinded! For three (12%) patient blinded, six (25%) therapy, four (17%) therapist, one (4%) other caregivers; two (8%) the outcome assessor; zero (0%) data analyst, zero (0%) manuscript writer.
Reporting/analysis: One (4%) checked statistical assumptions, 23 (96%) provided baseline data, not all for every patient randomized. Twenty-one (88%) provided *P* values for group comparisons, four (17%) provided confidence intervals and zero (0%) provided numbers needed to treat. One (4%) specified subgroups in advance [6], six (25%) adjusted for baseline differences as one of the reported analyses. Four (17%) stated statistical software, but not version, zero (0%) provided the computer used for analyses.
Imputation: Seventeen (71%) had missing data, yet one (4%) mentioned using last observation carried forward, zero (0%) used multiple imputation and zero (0%) mentioned impact on study conclusions [5]. Two (8%) provided a flowchart as suggested by CONSORT [7,8].
Conclusion: Lupus trials did not report many of the methodological criteria that give papers credibility and validity to the study being reported. Reporting should be improved in future reports of studies of patients with lupus and related health problems. Possibly using the CONSORT checksheets would help make lupus papers more credible [7,8].
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A55

Development validation and reliability of the Systemic Lupus Erythematosus Disease Activity Index 2000 Responder Index-50

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Background: The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) is a global disease activity index composed of 24 descriptors reflecting nine organ systems. The SLEDAI-2K measures only complete recovery in active descriptors on follow-up visits. Thus, an improvement in the SLEDAI-2K descriptors is captured when a manifestation has completely resolved. The aims were: to develop and validate an outcome measure, the SLEDAI-2K Responder Index-50 (S2K RI-50), able to capture partial improvement, ≥50%, in each of the active descriptors at subsequent visits; to test the reliability of S2K RI-50; and to evaluate S2K RI-50 sensitivity to response at 6 and 12 months.
Methods: The S2K RI-50 was constructed based on SLEDAI-2K. New definitions for all descriptors were generated along with criteria to identify 50% improvement. The S2K RI-50 Data Retrieval Form standardizes the documentation of the descriptors. From September 2009 to April 2011, all lupus patients seen at the Lupus Clinic were assessed at baseline and follow-up visits using SLEDAI-2K, S2K RI-50 and physician global assessment (visual analog scale online and Likert scale). Concurrent construct validity: correlation of the S2K RI-50 was determined using the Likert scale as the external construct. Inter-rater/intra-rater reliability (intraclass correlation coefficient (ICC)): 40 patient scenarios with baseline and follow-up visits derived from real patients were developed. Ten rheumatologists scored the profiles with the SLEDAI-2K and S2K RI-50, on two occasions 2 weeks apart. Sensitivity to response: responders using the SLEDAI-2K, S2K RI-50 and SLE Responder Index (SRI) were identified at 6 and 12 months among 103 consecutive active patients. Patients defined as S2K RI-50 responders were compared with SRI responders; considered the gold standard.
Results: The S2K RI-50 and its Data Retrieval Form were developed. The initial validation on 141 patients showed that S2K RI-50 has construct validity (correlated with Likert scale; $r = 0.48$; $P < 0.0001$). The S2K RI-50 is reliable and the ICC for inter-rater/intra-rater ranged from 0.86 to 1.00. The percentage of responders at 6 and 12 months was 44% and 51% by SLEDAI-2K and 43% and 51% by SRI respectively. The percentage of S2K RI-50 responders at 6 and 12 months was higher at 51% and 58% respectively.
Conclusion: The S2K RI-50 is novel valid and reliable responder index able to identify patients with partial, ≥50% improvement. The S2K RI-50 identified more responders as compared with the SLEDAI-2K and SRI at the 6 and 12 month period. The S2K RI-50 can be used independently to identify patients with clinically important improvement.
Acknowledgements: The authors acknowledge all who participated in these studies.

A56

Clinical and immunological response to pneumococcal vaccination in pediatric systemic lupus erythematosus

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Background: Sepsis is a leading cause of mortality in pediatric SLE with increased susceptibility to *Streptococcus pneumoniae* infection. We routinely vaccinate with the 23-valent pneumococcal polysaccharide

vaccine (PPV) to protect against infection. Response to vaccination may vary due to disease-specific factors and immunosuppressant use. The appropriate time to assess antibody response to PPV is unknown. Our study objectives were to describe the clinical and immunological response to PPV in pediatric SLE and to determine predictors of decline in immunogenicity after PPV.

Methods: We evaluated 54 pediatric SLE subjects who received the PPV at diagnosis with vaccine titers obtained at varying intervals. Change in disease activity after vaccination was assessed by the SLE Disease Activity Index (SLEDAI). A positive antibody response was defined as ≥7 of the 14 titers measured having a value ≥1 μg/ml. We used Cox proportional hazards to evaluate factors associated with lack of immunological response to PPV.

Results: The majority of the cohort were female (79%) and 52% were Hispanic ethnicity. Nephritis (74%), cytopenias (57%), and arthritis (50%) were the most common clinical features at baseline presentation. In the month prior to vaccination, 54% of the cohort received pulse methylprednisolone, and 20% received cyclophosphamide or mycophenolate mofetil. There was no change in the median SLEDAI score after vaccination (8 vs. 6, $P = 0.2$). One subject experienced an adverse reaction after initial vaccination, and two subjects developed severe pneumococcal disease despite vaccination. After initial vaccination, 59% of subjects did not achieve protective titers. The median time to inadequate response after initial vaccine was 0.73 years (0.16 to 3.1). In unadjusted models, age (HR = 1.2, $P = 0.02$, 95% CI = 1.02 to 1.4) and hydroxychloroquine use (HR = 2.4, $P = 0.05$, 95% CI = 1.0 to 5.7) were associated with a decreased response. However, after adjustment for age, sex, and ethnicity, disease characteristics and medication use were not associated with lack of immunologic response to PPV.

Conclusion: PPV was well tolerated in our cohort, but the majority of subjects failed to demonstrate adequate immunologic response to initial vaccination. Disease characteristics and medication use did not explain the lack of response. Pneumococcal infection may occur despite vaccination. PPV vaccination is recommended for patients with pediatric SLE but future studies in larger cohorts are needed to delineate risk factors for lack of immunogenicity.

A57

Role of anti-Ro autoantibodies in systemic lupus erythematosus patients with recurrent myositis

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Arthritis Research & Therapy 2012, **14(Suppl 3):**A57

Background: Anti-SSA (Ro) autoantibodies have been detected in primary Sjögren's syndrome as well as in patients with other systemic autoimmune diseases including systemic lupus erythematosus (SLE). Recent studies have suggested a possible association of anti-Ro antibodies in patients with systemic sclerosis and myositis [1]. These autoantibodies have also been detected in patients with the anti-synthetase antibody syndrome [2]. We investigated the association of anti-Ro antibodies in SLE patients with myositis.

Methods: In this retrospective study, we reviewed the records of 160 patients with SLE followed in the lupus clinic at LSU Health Sciences Center, Shreveport from 2000 to August 2012.

Results: We identified 16 patients (10%) with myositis and SLE. Patients with possible secondary cause of myositis, such as thyroid disease and drug, were excluded. All of the patients were females. Fourteen patients (87.5%) were African Americans and two (12.5%) were Caucasians with ages that ranged from 15 to 69 years. Among these 16 patients, 12 (75%) were found to have anti-Ro autoantibodies. The remaining four patients were not tested for anti-Ro. Five patients (41.7%) with higher levels of anti-Ro antibodies at the beginning of their disease experienced recurrences of the myositis. Average anti-Ro was compared between patients with and without recurrences to determine association between myositis recurrence and anti-Ro. A nonparametric test (Wilcoxon rank sum test) was used to do the comparison due the observed non-normal distribution of anti-Ro and the small sample sizes (five and seven) for the two groups compared. Mean ± SD for the recurrence and nonrecurrence groups was 470.4 ± 347.3 and 153.5 ± 113.4, respectively. Using the Wilcoxon rank sum test, the P value for the comparison was 0.06. This was not significant at the 5% level but significant at the 7% level ($P < 0.07$). The observed nonsignificance at the 5% level was due to the small sizes (five and seven) for the two groups compared

Conclusion: The observed results show possible association between myositis recurrence and anti-Ro antibody level. Prospective studies are required to further investigate this association. Myositis in SLE may be under-reported secondary to a lack of awareness of this association, which precludes the simultaneous testing of these autoantibodies along with muscle enzymes. This evaluation is vital during the beginning of disease and with flares because the ongoing muscle damage may be masked and undertreated by the subsequent therapy.

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A58

Variation in renal biopsy and medication prescribing practices among pediatric Medicaid patients with lupus nephritis prior to end-stage renal disease in the US, 2000 to 2004

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Background: Unequal medical care may contribute to striking socio-demographic disparities seen in outcomes for children with lupus nephritis. Medicaid is the US federal-state program providing health insurance to low-income children and parents. We investigated US nationwide variation in renal biopsies and medication prescriptions for children with lupus nephritis enrolled in Medicaid, 2000 to 2004, in the months preceding end-stage renal disease (ESRD).

Methods: We identified all children aged 3 to <18 years with SLE (≥3 ICD-9 codes of 710.0, each >30 days apart) in the Medicaid Analytic eXtract (MAX) from 2000 to 2004, which contains outpatient and inpatient Medicaid claims for enrollees in 47 US states and the District of Columbia. These data were linked to the US Renal Data System, with information on essentially all ESRD patients in the US, for the same years. We compared frequencies of renal biopsies, and prescription of corticosteroids, hydroxychloroquine (HCQ) and immunosuppressants (mycophenylate mofetil (MMF), cyclophosphamide (CYC), cyclosporine, azathioprine (AZA), tacrolimus), across categories of sex, race/ethnicity, socioeconomic status (SES), US region of residence, residence in a designated Health Professional Shortage Area (HPSA), and quartiles of pediatric rheumatologist number in state of residence. We tested for differences across categories using chi-squared and Fisher's exact tests, and applied the Cochrane Armitage test for trend.

Results: Of the 254 pediatric lupus nephritis patients who developed ESRD, the mean age was 14.2 (± 2.4) years; 72% were female, 61% were African American and 19% were Hispanic. The mean time from first SLE claim to ESRD was 3.8 (± 2.1) years. A total of 46% had at least one renal biopsy preceding ESRD. More children in the lower quartiles of SES and the higher quartiles of rheumatologist number per state, received a biopsy (P trend < 0.05) (Table 1). Ninety-one percent of children were prescribed steroids at some time preceding ESRD, 63% were prescribed HCQ and 66% any other immunosuppressant, 50% of whom were prescribed MMF, 30% AZA and 14% CYC. We observed variation in prescribed steroids across region of residence, HCQ and immunosuppressant across race (more non-White patients prescribed both medications), and a greater proportion of patients prescribed HCQ in states with a higher number of rheumatologists per state.

Conclusion: We observed significant differences in the proportion of children who had received renal biopsies across categories of SES and rheumatologist number per state, as well as marked differences in medication prescribing across categories race, SES, regions of residence and rheumatologist number in state of residence.

Table 1(abstract A58) Proportion of pediatric patients with lupus nephritis-associated ESRD who received renal biopsies and medications prior to ESRD

		Renal biopsy		Corticosteroids		HCQ		Immunosuppressants	
		n (%)	*P* value[a]	*n* (%)	*P* value[a]	*n* (%)	*P* value[a]	*n* (%)	*P* value[a]
Total	254								
Sex									
Female	182	89 (49)	0.15	167 (92)	0.47	117 (64)	0.38	121 (66)	0.85
Male	72	28 (39)		64 (89)		42 (58)		47 (65)	
Race/ethnicity									
White	25	14 (56)	0.52	22 (88)	0.19	11 (44)	1×10^6	13 (52)	0.02
Black	155	74 (48)		139 (90)		91 (59)		103 (66)	
Hispanic	48	21 (44)		47 (98)		44 (92)		34 (71)	
Asian	17	-		16 (94)		-		15 (88)	
Native	-	-		-		-		-	
SES group									
Quartile 1 - lowest	63	37 (59)	0.02[b]	60 (95)	0.21[b]	36 (57)	0.33[b]	42 (67)	0.95[b]
Quartile 2	63	27 (43)		56 (89)		41 (65)		42 (67)	
Quartile 3	63	29 (46)		58 (92)		39 (62)		42 (67)	
Quartile 4 - highest	64	23 (36)		56 (88)		43 (67)		42 (66)	
Region of residence									
Northeast	35	10 (29)	0.09	30 (86)	0.004	22 (63)	0.01	30 (58)	0.53
Midwest	52	26 (50)		43 (83)		23 (44)		23 (66)	
South	123	63 (51)		120 (98)		81 (66)		85 (69)	
West	44	18 (41)		38 (86)		33 (75)		30 (68)	
Residence in health professional shortage area									
Not HPSA	13	-	0.78	13 (100)	0.48	-	0.09	11 (85)	0.12
HPSA	240	110 (46)		217 (90)		151 (63)		157 (65)	
Pediatric rheumatologist per state									
Quartile 1 - lowest	63	38 (60)	0.0002[b]	59 (94)	0.24[b]	35 (56)	0.02[b]	43 (68)	0.50[b]
Quartile 2	64	34 (53)		62 (97)		37 (58)		46 (72)	
Quartile 3	72	30 (42)		63 (88)		46 (64)		42 (58)	
Quartile 4 - highest	55	15 (27)		47 (85)		41 (75)		37 (67)	

–, cell sizes under 11 have been suppressed in accordance with Centers for Medicare and Medicaid Services policy to protect privacy. [a]*P* value for chi-square tests and Fisher's exact tests for small cell counts. [b]*P* value for Cochran-Armitage Trend tests.

A59

Hospitalizations in patients with systemic lupus erythematosus: updated analyses from 2006 to 2011

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Background: Health resource use is believed to be significant in patients with systemic lupus erythematosus (SLE), but there is a lack of data especially in Canadian patients regarding the reasons why persons with SLE require hospitalization and the rates of hospitalization compared with the general population. Our objective was to provide recent estimates for hospitalization rates and reasons for admission, in a clinical SLE cohort.

Methods: We evaluated data from patients with SLE followed at the McGill University Health Center Lupus Clinic. Information on disease activity, drug exposure, health outcomes, and hospitalizations by self-report were collected from annual research visits. The hospitalization rates of the SLE patients were generated. We compared this with the Canadian general population by calculating the standardized incidence ratio (SIR), which represents the ratio of the number of events observed in the SLE cohort to the number of events that would be expected based on the age-specific and sex-specific Canadian general population hospitalization rates.

Results: Over the interval studied, 350 patients (325 female, 25 male) provided 1,261 person-years of follow-up. There were 163 reported admissions with an incidence of 12.8 hospitalizations per 100 person-years (12.4 in females, 19.5 in males). SLE-related causes (for example, flares) accounted for the highest proportion of hospitalization (22.7%), followed by infections (20.2%), surgery (14.7%), childbirth (11.7%) and cardiovascular reasons (11.0%). The overall SIR was 1.73 (95% CI = 1.48 to 2.02). Stratified by sex, the SIR was 2.87 (95% CI = 1.67 to 4.60) for males and 1.39 (95% CI = 1.18 to 1.64) for females. However, stratifying further by age, female SLE patients aged >65 actually underwent fewer hospitalizations than expected, based on age/sex-specific general population rates (SIR = 0.35; 95% CI = 0.17 to 0.64). In male SLE patients over 65, there were no hospitalizations (compared with 1.48 expected events), and the confidence interval (95% CI = 0.0 to 2.49) around the SIR was very imprecise in this demographic, due to the relatively low number of older males in our cohort.

Conclusion: We documented high rates of hospitalization in our SLE patients, particularly for male patients. Hospitalizations were often due to SLE-related reasons and infections. Female SLE patients over the age of 65 were shown to have a much lower hospitalization rate compared with the general population, which may be due to a survivorship bias. Further work on the variables affecting hospitalizations in SLE patients is in progress.

A60

B-cell receptor signaling studies in patients with lupus: preliminary results

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Background: There is significant evidence from murine and human genomic studies that B-cell receptor (BCR) signaling abnormalities are potential factors in the pathogenesis of lupus. However, data on signaling deviations in lupus patients are scant. We have undertaken a project to comprehensively study BCR signaling deviations in lupus patients.
Methods: Peripheral blood mononuclear cells will be isolated and frozen. B cells will be stimulated with F(ab')2 anti-IgM and anti-IgG. Using eight-parameter flow cytometry, we will determine signaling amplitude, as measured by phosphorylated ERK1/2, in IgG memory cells ($CD20^{+}IgG^{+}$), mature naïve ($CD20^{+}$,$CD27^{low}$,$CD38^{low}$), transitional ($CD20^{+}$,$CD38^{high}$, $CD10^{high}$), B1 ($CD20^{+}$,$CD27^{high}$) and IgM memory B cells ($CD20^{+}$,IgM^{+}, $CD27^{high}$,$CD86^{high}$). We will measure pERK levels at baseline (time 0), 1 minute (early signal), 5 minutes (peak signal) and 15 minutes (late signal). We will establish normal parameters by studying BCR signaling in 100 nonautoimmune individuals. We will then determine the prevalence of low and high BCR signaling deviations in 100 lupus patients. Signaling deviations will be correlated with clinical data.
Preliminary results: We have optimized a protocol that enables us to identify each of the above B-cell subsets while assaying phospho-ERK levels. Results from four lupus patients indicate that this protocol is able to identify signaling differences between individuals with lupus. See Figure 1.
Conclusion: The extent to which signaling deviations contribute to autoimmunity in patients with lupus remains to be determined. By elaborating a robust method to assay BCR signaling, we hope not only to measure this pathogenic factor but also to answer fundamental questions, such as whether diminished BCR signaling correlates with a broader autoimmune phenotype, as would be postulated by the impaired negative selection that is caused by diminished signaling. In the future, we will aim to convert signaling studies from research tools to clinical tools.

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Lupus cardiomyopathy: a reversible form of left ventricular dysfunction

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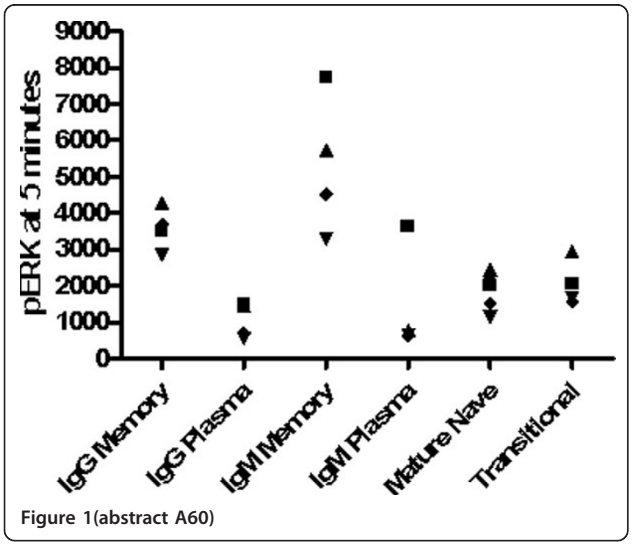

Figure 1(abstract A60)

Background: Myocarditis has been reported to be a common postmortem finding of systemic lupus erythematosus (SLE) patients. However, most case reports on SLE cardiomyopathy have not found myocarditis on biopsy. Stress-related cardiomyopathies result in reversible left ventricular (LV) dysfunction. The purpose of this study is to characterize the nature and course of LV dysfunction in SLE patients.
Methods: We evaluated a large cohort of SLE patients hospitalized at Cedars Sinai Medical Center between 28 August 2001 and 30 October 2010. Patients were included in the study that met American College of Rheumatology criteria for SLE, had an erythrocyte sedimentation rate and high sensitivity C-reactive protein performed, and had an echocardiogram with ejection fraction (EF) <45% during index hospitalization. Admission data, medications, and echocardiograms were reviewed.
Results: Five-hundred and twenty-six SLE patients were surveyed, of which 15 patients met all study inclusion criteria with LVEF ranging from 15 to 45%, mean 33 ± 9.8%, Twelve of 15 patients demonstrated a reversal of acute cardiomyopathy, showing an improvement in LVEF from 10 to 40%, mean 23.4 ± 9%. Twelve patients had generalized LV hypokinesis. Two patients underwent coronary angiography and had no obstructive coronary lesions. One patient also underwent cardiac biopsy, which did not show any evidence of myocarditis. Of the three patients whose cardiomyopathy did not reverse, all died due to their underlying medical illness.
Conclusion: This is the first report to describe a reversible cardiomyopathy in SLE patients. The pattern of wall motion abnormalities and its reversibility is more indicative of a stress-related cardiomyopathy syndrome than being the result of myocarditis.

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Prevalence of angina in patients with systemic lupus erythematosus

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Background: The study of coronary artery disease (CAD) in systemic lupus erythematosus (SLE) has become increasingly important with evidence indicating a relationship between cardiovascular disease outcome and chronic inflammatory conditions [1]. Since anginal chest pain (CP) is a frequent clinical symptom in patients with CAD, it is important to estimate its frequency among SLE patients. The Rose angina questionnaire and the Diamond-Forrester index are widely used tools to assess angina in the general population but have not previously been used to measure angina prevalence in SLE [2,3]. We conducted a pilot study on the prevalence of self-reported CP in patients with SLE.
Methods: Informed consent was obtained from consecutive adult SLE patients presenting to a rheumatology practice between January 2010 and April 2010. All patients were assessed by American College of Rheumatology criteria for SLE and confirmed to have SLE by the rheumatologist and by chart review. Two self-administered questionnaires were completed on a single visit. The Rose angina questionnaire assessed whether patients have ever had CP or angina and the Diamond-Forrester index ascertained whether patients were experiencing current angina. For data management and statistical analysis, SAS 9.1 and STATA 10 were employed. The confidence intervals represent Wald confidence intervals of the proportion ± 1.96× standard error truncated to zero or one in cases where the limits have been outside that range. Atypical or typical angina by the Diamond index was considered angina.
Results: A total of 150 SLE subjects were enrolled (94% female, mean age 43 ± 13 years, mean disease duration 12 ± 9 years). Ninety-six subjects (65.8%; 95% CI = 58.0 to 73.5) indicated they had ever experienced CP. A history of angina by Rose angina questionnaire was reported in 18 patients (12.3%; 95% CI = 6.9 to 17.7). Thirty-one (21.2%; 95% CI = 14.5 to 27.9) patients indicated they were experiencing current CP and were administered the Diamond index; 12 (8.2%; 95% CI = 3.7 12.7) subjects had current angina by Diamond index, including two patients (1.4%; 95% CI = 0 to 3.3) with typical angina. No relationship was found between age or disease duration and scores on either questionnaire.
Conclusion: Our data indicate a 12.3% prevalence of ever having had angina, and an 8.2% prevalence of current angina. A recent meta-analysis

of worldwide responses to the Rose angina questionnaire found the population weighted mean of angina prevalence to be 6.7% in women and 5.7% in men [4]. It appears that the prevalence of angina history in SLE patients is approximately twice that in the general population.

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Functional genetic polymorphisms in ILT3 are associated with decreased surface expression on dendritic cells and increased serum cytokines in lupus patients

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Background: Hyperactivity of the type I interferon (IFN) pathway is involved in the pathogenesis of systemic lupus erythematosus (SLE). Immunoglobulin-like transcript (ILT3) is an immunohibitory transmembrane molecule that is induced by type I IFNs. ILT3 is expressed by plasmacytoid dendritic cells (PDCs), monocytoid dendritic cells (MDCs), and monocytes/macrophages. Given the pathogenic role of IFN in SLE, we hypothesized that the IFN-induced immunosuppressive ILT3 receptor may be dysfunctional in human SLE.

Methods: In total, 132 European-derived and 79 Hispanic-American SLE patients were genotyped for two coding-change SNPs predicted to interfere with protein folding in ILT3 (rs11540761 and rs1048801). One hundred and sixteen control DNA samples and sera from healthy controls were also studied. We detected associations between ILT3 genotype and serum cytokine profiles. ILT3 expression levels on PDCs and MDCs from 18 patients and 10 controls were studied by flow cytometry.

Results: The rs11540761 SNP in the extracellular region was associated with decreased cell surface expression of ILT3 on circulating MDCs and to a lesser extent PDCs in SLE patients. The cytoplasmically located rs1048801 SNP was not associated with a change in DC expression of ILT3. Both SNPs were significantly and independently associated with increased levels of serum type I IFN activity in SLE patients. The rs1048801 SNP was also associated with increased serum levels of TNFα.

Conclusion: Loss-of-function polymorphisms in ILT3 are associated with increased inflammatory cytokine levels in SLE, supporting a biological role for ILT3 in SLE.

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Nonlymphoma hematological malignancies in systemic lupus erythematosus

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Objective: To describe nonlymphoma hematological malignancies in systemic lupus erythematosus (SLE).

Methods: An international, multisite (n = 28) SLE cohort was linked to regional tumor registries. We examined the types of nonlymphoma hematological cancers occurring after SLE diagnosis, and their demographic characteristics, including sex, race/ethnicity and age at time of cancer diagnosis.

Results: A total of 15,980 patients were observed for an average of 7.5 person-years. Of these, 90% were female and the majority was Caucasian. Based on age-matched general population cancer rates, the standardized incidence ratio for hematological cancers after SLE onset was 2.9 in females (95% CI = 2.3 to 3.6) and 3.6 in males (95% CI = 2.2 to 5.5). A total of 115 hematological cancers occurred: 82 were lymphoma (75 non-Hodgkin's, seven Hodgkin's), and 33 were nonlymphoma.

Of the 33 nonlymphoma cases, 13 were of lymphoid lineage: multiple myeloma (MM, n = 5), plasmacytoma (n = 3), B-cell chronic lymphocytic leukemia (B-CLL, n = 3), lymphocytic leukemia (n = 1), and precursor cell lymphoblastic leukemia (n = 1). The remaining 20 cases were of myeloid lineage: myelodysplastic syndrome (MDS, n = 7), acute myeloid leukemia (AML, n = 7), chronic myeloid leukemia (CML, n = 2), and four unspecified leukemias.

All lymphoid malignancies occurred in female Caucasians, except for plasma cell neoplasms, where 4/5 MM cases and 1/3 plasmacytoma cases occurred in blacks (the others being Asian and Caucasian). At the time of MM diagnosis in SLE, the median age was 49 years (range 45 to 57), while for the three plasmacytoma SLE cases the median age was 35 years (range 25 to 62). In the female general population, median age at onset is 70 years for MM [1] and 55 years for plasmacytomas [2]. The median age of SLE subjects at B-CLL onset was 65 years (range 58 to 83), similar to the female general population (74 years).

Of 20 myeloid malignancies, three (15%) occurred in males, and six of the 20 myeloid malignancies (30%) occurred in blacks. All seven AML cases were female, with median age at AML diagnosis of 48 years (range 34 to 72), versus 66 years in the female general population. The seven MDS cases (six females) occurred at a median age of 48 years (range 36 to 59), versus 76 years in the general population. The ages at time of diagnosis for the two CML cases (one female) were similar to the general population median (65 years).

Conclusion: In our SLE cohort, the most common nonlymphoma hematological malignancies observed were myeloid types (MDS and AML). This is in contrast to the general population, where lymphoid types are three times more common than myeloid [3]. Most (80%) MM cases in our SLE cohort occurred in blacks. Most of our nonlymphoma hematological malignancy cases were younger than general population median age of onset, although this could simply reflect our cohort demographics.

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