antibodies to a gene expressed on demethylated but not normal T cells may treat lupus flares.

**Footnotes**

Dept of Medicine, University of Michigan, Ann Arbor MI

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**GG-02**

**EPIGENETIC REPROGRAMMING IN NAÏVE CD4+ T CELLS FAVOURING T CELL ACTIVATION AND NON-TH1 EFFECTOR T CELL IMMUNE RESPONSE AS AN EARLY EVENT IN LUPUS FLARES**

Patrick Coit, Mikhail G Dozmorov, Joan T Merrill, W Joseph McCune, Kathleen Maksimowicz-McKinnon, Jonathan D Wren, Amr H Sawalha*

1Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA; 2Department of Biostatistics, Virginia Commonwealth University, Richmond, Virginia, USA; 3Clinical Pharmacology Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA; 4Division of Rheumatology, Henry Ford Health System, Detroit, Michigan, USA; 5Arthritis and Clinical Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA; 6Department of Biochemistry and Molecular Biology, The University of Oklahoma Health Sciences Centre, Oklahoma City, Oklahoma, USA; 7Centre for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA

These two authors equally contributed to this work

**Background**

Systemic lupus erythematosus is a relapsing autoimmune disease that affects multiple organ systems. T cells play an important role in the pathogenesis of lupus, however, early T cell events triggering disease flares are incompletely understood. We studied DNA methylation in naïve CD4+ T cells from lupus patients to determine if epigenetic remodelling in CD4+ T cells is an early event in lupus flares.

**Materials and methods**

A total of 74 lupus patients with disease activity ranging from 0–18 as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) were included in this study. Naïve CD4+ T cells were isolated from peripheral blood samples and DNA extracted for genome-wide methylation assessment. RNA was also extracted from a subset of patients to determine the relationship between epigenetic changes and transcriptional activity using RNA sequencing and microRNA arrays.

**Results**

We demonstrate that naïve CD4+ T cells in lupus undergo an epigenetic pro-inflammatory shift implicating effector T cell responses in lupus flare. This epigenetic landscape change occurs without expression changes of corresponding genes, and poises naïve CD4+ T cells for Th2, Th17, and Th1 immune responses, and opposes inhibitory TGF-β signalling. Bioinformatics analyses indicate that the epigenetic modulator EZH2 might be playing an important role in shifting the epigenetic landscape with increased disease activity in lupus naïve CD4+ T cells. Further, the expression of miR26a and miR101, which are sensitive to glucose availability and target EZH2, negatively correlated with disease activity in lupus patients.

**Conclusion**

An epigenetic landscape shift in naïve CD4+ T cells that favours T cell activation and non-Th1 immune responses precedes transcriptional activity and correlates with lupus activity. A role for EZH2 dysregulation in triggering lupus flares warrants further investigation. The proposed T cell epigenetic model of disease flare in lupus patients is depicted in Figure 1.

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**GG-03**

**STAT1-STAT4 ASSOCIATION WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)**

Zubin Patel, Matt Weirauch, Leah C Kottyan, John B Harley*

1University of Cincinnati and the Centre for Autoimmune Genomics and Aetiology (CAGE), Cincinnati Children’s Hospital Medical Centre, Cincinnati, OH; 2US Department of Veterans Affairs Medical Centre, Cincinnati, OH

**Background**

Signal Transduction and Activation of Transcription (STAT) transcription factors are evolutionarily ancient, mediating signals from the cytoplasm to the nucleus in eukaryotic life for the past 400 million years. The STAT protein sits quiescent in the cytoplasm until phosphorylated whereupon it dimerizes with another STAT protein. The phosphorylated STAT dimer is then transported to the nucleus and becomes a transcription factor activating or suppressing gene expression. The STAT1–STAT4 dimerizes with STAT4 to form the classical Th1/Th2 transcription factor, and with STAT1 to form the classical Th17/Th1 transcription factor. Further, the Th17/Th1 transcription factor isotypes are also expressed in Tregs.

**Results**

An association between STAT1–STAT4 expression and disease activity in lupus patients was observed. A statistical model predicting disease activity included STAT1 and STAT4.

**Conclusion**

A role for STAT1–STAT4 association in the pathogenesis of lupus warrants further investigation.

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**Abstract GG-02 Figure 1**

T cell epigenetic model of disease flares in lupus
locus has powerful common variant associations with SLE risk with odds ratio $= 1.5$ and $p < 10^{-20}$ in all human ancestries and also has an association, though not necessarily identical to SLE, with rheumatoid arthritis, primary biliary cirrhosis, Behçet’s Disease, Sjögren’s syndrome, progressive systemic sclerosis, and type 1 diabetes.

Methods Genome wide association studies of SLE, Bayesian and frequentist fine mapping methods, DNA affinity purification assays, and electrophoretic mobility shift assays.

Results We are attempting to identify the causal variants and determine the mechanism for SLE disease risk at this locus. Our data suggest that the risk haplotype alters the expression of mRNA from both STAT1 and STAT4. Application of frequentist and Bayesian methods restrict the plausibly causal variants to four possibilities in introns 4 and 5 of STAT4 under the assumption that the association observed across human ancestries is being driven by the same causal variants. Three of these four polymorphisms are predicted to alter the binding of a specific transcription factor, leading to the hypothesis that the same transcription factor is operating at multiple sites in a risk haplotype.

We have data suggesting differential and allele preferential binding of the transcription factor at one variant with evaluation of the others in process. This may possibly be the first discovered example of the phenomenon of multiple transcription factor binding on multiple variants of a risk haplotype.

Conclusion In general, genome wide association studies (GWAs) provide powerful evidence of the presence of a genetic variation altering phenotype risk without revealing what the specific responsible variant is among those in a statistical dead heat for causation, in which cell type(s) or stage(s) of differentiation in which the risk difference is relevant, or what the molecular mechanism might be. We have work underway to reveal these details for lupus loci, initially concentrating on IRF5, STAT1-STAT4, and ET31. The STAT1-STAT4 association with SLE can be isolated to involve only a few variants, which are predicted to have curiously similar transcription factor binding behaviour.

**Background** Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with a strong genetic component. Dozens of SLE-associated loci have been identified by genome-wide associated studies (GWAS) and included in the ImmunoChip for fine-mapping.

**Materials and methods** Using ImmunoChip, we assessed case-control subjects including Chinese, European Americans (EA) and African Americans (AA) for association with SLE. Subsequently, we carried out trans-ancestral mapping and resequenced the complex $GTF2IRD1$-$GTF2I$-$NCF1$ region on 7q11.23 rather than SLE-associated GWAS loci. This association was confirmed in EA ($OR = 1.37, P = 2.5 \times 10^{-5}$) but not in AA. By trans-ancestral mapping and sequencing, we identified R90H of NCF1, a neighbouring gene of GTF2I encoding the p47phox subunit of NADPH oxidase, as a highly plausible causal variant.

R90H was associated with SLE in East Asians ($OR = 3.47, P_{meta} = 3.0 \times 10^{-10}$), EA ($OR = 2.11, P_{meta} = 7.0 \times 10^{-8}$) and AA ($OR = 1.91, P = 7.2 \times 10^{-5}$), and in conditional test R90H eliminated SLE-associated signals within the $GTF2IRD1$-$GTF2I$ region including rs73366469. Furthermore, R90H was dose-dependently associated with early age of onset in Korean ($P = 0.011$) and EA ($P = 0.012$) patients with SLE. In addition to SLE, R90H was associated with seropositive rheumatoid arthritis (RA) in Koreans ($OR = 1.66, P = 1.2 \times 10^{-5}$) and primary Sjögren’s syndrome (SS) in EA ($OR = 1.72, P = 5.8 \times 10^{-5}$). The conserved arginine 90 to histidine substitution located in the PX-binding domain of p47phox is predicted deleterious, which is supported by a report showing R90H results in reduced reactive oxygen species (ROS) production.

**Conclusions** We identified R90H of NCF1 as a novel risk variant for multiple autoimmune diseases, highlighting the pathogenic role of reduced ROS production in developing autoimmune diseases.