II-21 INTERFERING WITH INTERFERON IN LUPUS: HITTING THE SWEET SPOT WITH CNTO 6358

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Background The type I interferon (IFN-I) family of cytokines is thought to play a central role in the pathogenesis of systemic lupus erythematosus (SLE). Advanced glycation end products (AGEs) are prevalent in the Western diet. The accumulation of serum AGEs disrupts protein function, and interaction with its receptor induces production of reactive oxygen species and activation of vascular endothelial cells, leading to increased oxidative stress. Our hypothesis is that serum AGE levels, indicators of dietary habits, correlate with inflammation and potentially impact autoimmunity.

Materials and methods We evaluated clinical data and serum samples from 80 Gullah African American participants enrolled prospectively into the SLE in Gullah Health (SLEIGH) study. Of the 80 participants, 50 were patients with SLE and 30 were unaffected controls (15 related controls and 15 unrelated controls). Serum samples were assessed with the AGE Competitive ELISA kit from Cell Bio Labs. All samples were normalised to total protein concentration.

Results Overall there were no significant differences in mean AGE levels between SLE patients (2.8 mcg/mL ± 1.8), related controls (5.0 mcg/mL ± 3.1) or unrelated controls (1.2 mcg/mL ± 0.6). Obese patients (BMI ≥ 30) had significantly higher AGE levels than non-obese patients (p = 0.03), though there was no difference among controls. Smoking history was associated with higher AGE levels (p = 0.03). Although on average higher, AGE levels were not significantly associated with diabetes, hyperlipidemia, or stroke history. There was no difference in mean AGE levels with presence of hypertension or current corticosteroid use. Regression models demonstrated no significant influence of AGE level on patient or control status (OR 0.93, p = NS), including when adjusted for gender, age (in years) and BMI. Interestingly, among controls, ANA positivity significantly correlated with higher AGE levels (p = 0.01), when adjusted for age (years).

Conclusions Although there was no difference in AGE levels between SLE patients and controls, the AGE levels were higher with ANA positivity among controls. This finding suggests that serum AGE levels may play a role as a modifiable risk factor for autoimmunity and further study is warranted.

Genetics, Genomics and Epigenetics

II-22 ADVANCED GLYcation END PRODUCTS (AGEs) AND ASSOCIATION WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background Inhibiting DNA methylation in human or mouse CD4+ T cells causes overexpression of methylation sensitive genes including CD11a, CD70, CD40L and the killer-cell immunoglobulin like receptor (KIR) gene family, and the epigenetically altered murine cells are sufficient to cause lupus-like
autoimmunity in syngeneic mice. CD4+ T cells from patients with active lupus also have hypomethylated DNA and overexpress the same genes. Whether the methylation sensitive genes are expressed on different T cells or co-expressed on the same cell is unknown but important to determine, because antibodies to one of the proteins would deplete the subset if all are expressed on the same cell, providing a safer and more effective treatment than currently used medications. We have now used multicolor flow cytometry to test if all these genes are co-expressed on the same or different T cells, using CD4+ T cells experimentally demethylated in vitro and CD4+ T cells from patients with active lupus.

**Materials and methods** Peripheral blood mononuclear cells (PBMC) were isolated from healthy women and women with lupus by density gradient centrifugation. PBMC from healthy women were stimulated with PHA then treated for 72 hours with the Dnmt1 inhibitor 5-azacytidine (5-azaC). Lupus disease activity was determined using the SLEDAI. T cells stained with fluorochrome conjugated antibodies biotin-CD40L/ PECY7-avidin, APC-CD11a, Pacific Blue-CD3, PECy5-CD28, FITC-CD70, APCCy7CD4, and a “cocktail” of anti-KIR antibodies including PE-anti- KIR2DL4/CD158D, PE-anti- CD158b, PE-anti- CD158l, PE-anti-CD158b1/b2,j, and PE-anti- CD158a,h were analyzed using a FACS ARIA IIIu flow cytometer and FACSDiva software or an iCyte Synergy flow cytometer and WINLIST software.

**Results** The 5-azaC treated cells, but not untreated T cells, contained a CD3+CD4+CD28+CD11a hi KIR + CD70 + CD40L hi subset representing a range of 3–6% of the 5 AzaC-treated total CD4+ T cells. Similarly, PBMC from patients with active but not inactive lupus also contained a CD3+CD4+CD28+CD11a hi KIR + CD70 + CD40L hi subset representing a range of 3–6% of the 5 AzaC-treated total CD4+ T cells. Similarly, PBMC from patients with active but not inactive lupus also contained a CD3+CD4+CD28+CD11a hi KIR + CD70 + CD40L hi subset, and the size of the subset was directly proportional to disease activity (Figure 1).

**Conclusion** These results demonstrate that CD4+ T cells experimentally demethylated in vitro, and CD4+ T cells from women with active but not inactive lupus, contain a novel epigenetically altered subset that co-expresses the methylation sensitive genes. This subset may be a novel marker for lupus disease activity. Co-expression of the genes on the same cell also suggests that
antibodies to a gene expressed on demethylated but not normal T cells may treat lupus flares.

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**Abstracts**

**GG-02**

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

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**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 Tfh 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 predates 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)**

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**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...