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) and therapeutic approaches to down-modulate this pathway have demonstrated clinical efficacy. The IFN-I pathway is composed of multiple closely related IFN-α subtypes and single functional molecules for IFN-β, IFN-ϵ, IFN-κ and IFN-ω. Some members of the IFN-I family are thought to contribute to SLE pathogenesis, while others may be more relevant for host defense. We developed a fully-human monoclonal antibody (CNTO 6358) to selectively neutralise the predominant soluble IFN-Is expressed in SLE, while retaining the functions of IFN-β. Here we demonstrate *in vitro* bioactivity using endogenous SLE patient-derived IFN-I preparations, including immune complexes and serum/plasma from racially diverse SLE populations, providing evidence that the neutralisation and potency profile of CNTO 6358 may enable potent suppression of IFN-Is prevalent in SLE without suppression of other IFN-Is more essential for other host defense processes. We further describe an *in vitro* assay that may enable the prediction of responders and non-responders with CNTO 6358, providing a transformational framework for SLE precision medicine.

Materials and methods Pooled SLE serum and plasma or conditioned media from cells exposed to pooled SLE patient immune complexes or recombinant human IFN-Is were utilised as stimuli in an ISRE reporter gene assay (RGA) in the presence of CNTO 6358 or control. SLE patient whole blood was incubated *in vitro* for 24 hours in the presence or absence of CNTO 6358 and the impact of inhibitor treatment on gene expression was determined by RNA-Seq relative to untreated healthy donor samples.

Results IFN-I activity present in plasma and serum preparations from multiple SLE cohorts and activity present in conditioned media from PBMCs exposed to SLE patient immune complexes was neutralised to levels seen in healthy control samples. The *in vitro* addition of CNTO 6358 to blood from individual SLE patients enabled the identification of donors having robust normalisation of baseline elevated IFN-I signature gene expression and those having moderate to minimal transcriptional changes.

Conclusions CNTO 6358 exhibited potent neutralisation of multiple SLE patient-derived IFN-I preparations, demonstrating a bioactivity profile targeting the most prevalent IFN-I subtypes elevated in SLE patients, while preserving the functionality of other IFN-Is which may be more important for host defense. Furthermore, our *in vitro* assay and *in silico* methodologies may enable prediction of responders to our treatment, providing a transformational framework for SLE precision medicine.

II-22 ADVANCEDGLYCATION END PRODUCTS (AGES) AND ASSOCIATION WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background Oxidative stress plays a role in disease activity and premature atherosclerosis seen in patients with 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.

The cohort consisted of 50 patients (25 with no history of cardiovascular disease, and 25 with history of cardiovascular disease or renal disease) and 30 controls (15 related controls, 15 unrelated healthy controls). Student's T-test was used to compare AGE levels among SLE patients to controls. We used logistic regression models to examine predictors of autoantibody status and a nested case-control design to compare AGE levels between different disease subsets of patients.

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 not a 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

GG-01 CHARACTERISATION OF A $CD3^+CD4^+CD28^+CD11A^{HI}KIR^+CD70^+CD40L^{HI}$ T CELL SUBSET IN PATIENTS WITH ACTIVE LUPUS

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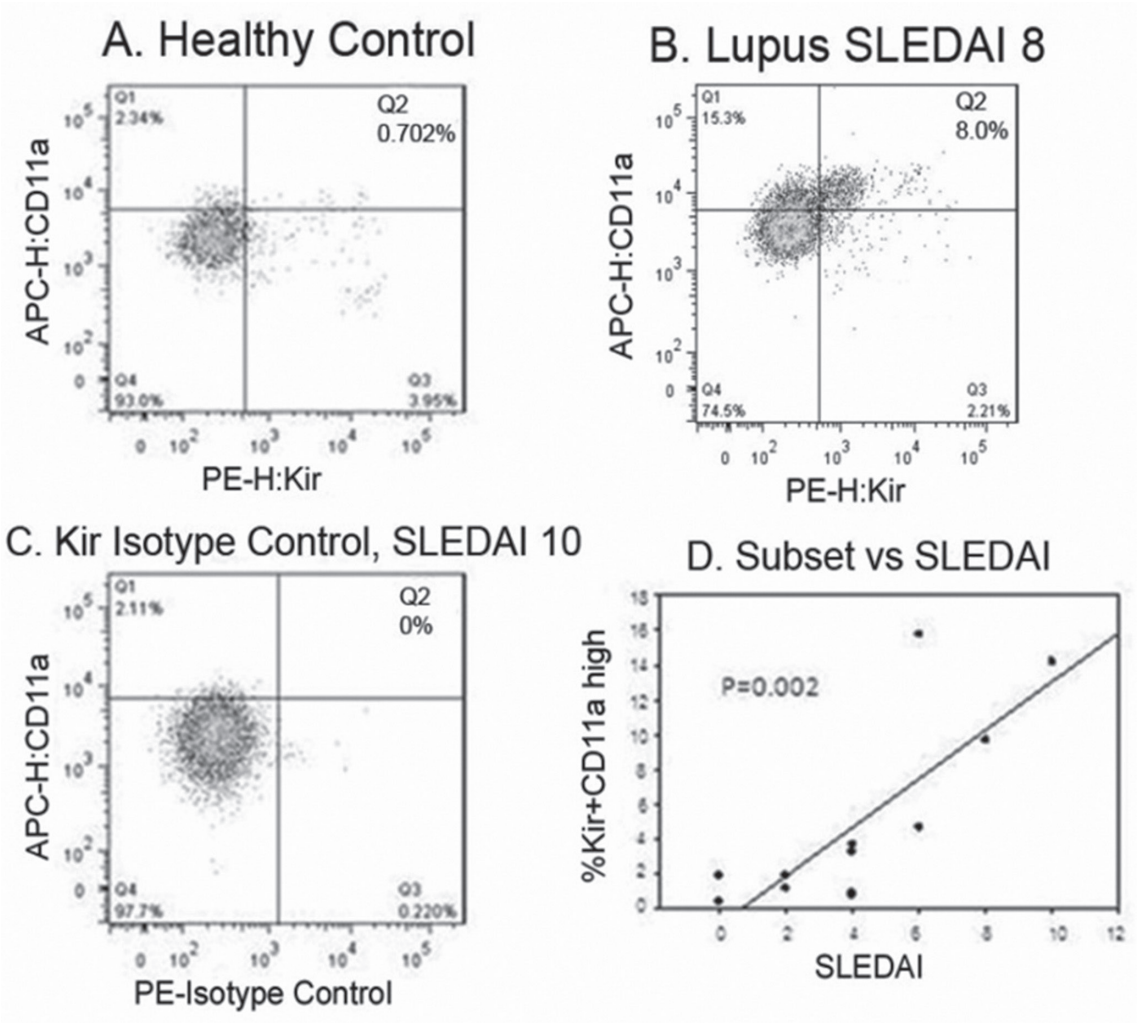
Background Inhibiting DNA methylation in human or mouse CD4+ T cells causes overexpression of methylation sensitive genes including CD11a, CD70, CD40L and the killer-cell immunoglobulin like receptor (KIR) gene family, and the epigenetically altered murine cells are sufficient to cause lupus-like



Abstract GG-01 Figure 1 PBMC from healthy female donors or lupus patients were stained with an antibody cocktail described in Methods and analyzed by flow cytometry. CD3+CD4+CD28+ cells were selected and CD11a and Kir measured. (A), untreated cells (representative of 5 healthy subjects); (B), one patient with a SLEDAI of 8; (C), PE-isotype control from the same patient in (D) with active (SLEDAI) disease; (D), percent of total CD3+CD4+CD28+ T cells that are also CD11ahigh and Kir+ versus lupus disease activity.

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 fluorchrome 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- CD158i, 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, 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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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

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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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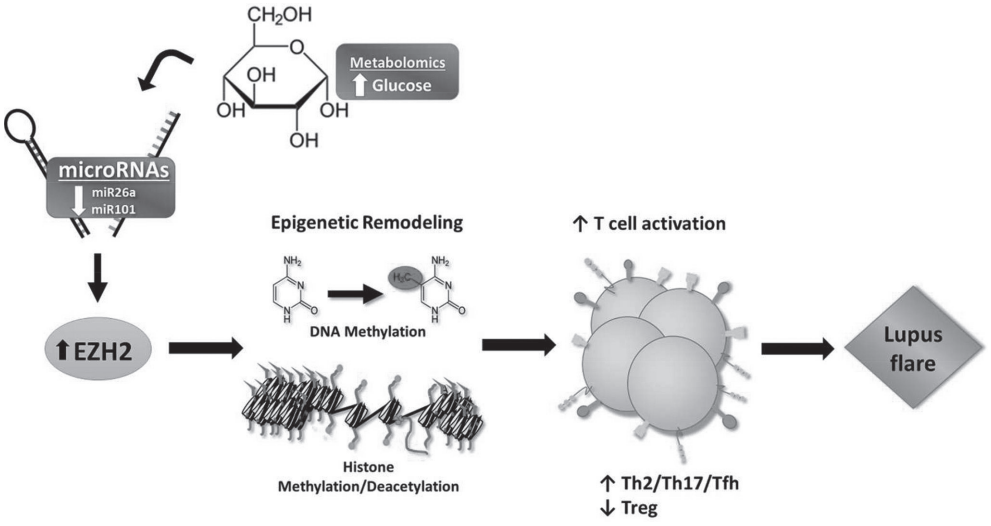
Background Signal Transduction and Activation of Transcription (STAT) transcription factors are evolutionarily ancient, mediating signals from the cytoplasm to the nucleus in eukaryotic life for the past 400 million years. The STAT protein sits quiescent in the cytoplasm until phosphorylated whereupon 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*



Abstract GG-02 Figure 1 T cell epigenetic model of disease flares in lupus