Our recent study has demonstrated induction of regulatory B cells (Bregs) by bacterial DNA in MRL/*lpr* mice leading to attenuation of SLE. This suggests a regulatory role for the gut bacteria in SLE development. However, the mechanism by which bacterial DNA induces Breg cell differentiation remains to be elucidated. Bacterial DNA contains unmethylated CpG motifs which are recognized by the innate immune molecule, toll-like receptor 9 (TLR9). We therefore hypothesize that gut bacteria-derived DNA induces Bregs in a TLR9-dependent manner, which in turn protects against lupus initiation. To test our hypothesis, TLR9 global knockout (*Tlr9*$^{-/-}$) MRL/*lpr* mice and their heterozygous (*Tlr9*$^{+/-}$) littermates were randomized into 2 groups per genotype. Beginning at 4 weeks of age, one group in each genotype received 80 µg *E. coli* double- stranded DNA (dsDNA) once a week by oral gavage for 4 consecutive weeks, whereas the other group received phosphate buffered saline (PBS) as control. Urine samples were collected before euthanasia whereas blood, spleen, lymph nodes, and kidneys were collected and processed following euthanasia at 15 weeks of age. *In vitro* culture treatment with bacterial DNA was used to investigate a TLR9-dependent, B cell-intrinsic or -extrinsic mechanism in Breg cell induction. Our data showed a trend in decrease of proteinuria, as well as sizes of spleen and lymph nodes, with bacterial DNA treatment in *Tlr9*$^{+/-}$ mice. Interestingly, serum levels of anti-dsDNA IgM, which has been shown to be protective against lupus, were significantly increased in bacterial DNA-treated *Tlr9*$^{+/-}$ mice, although the pathogenic anti-dsDNA IgG remained unchanged. Additionally, flow cytometry analysis of splenocytes showed an increasing trend in the percentage of interleukin-10 positive (IL-10^{+}) Bregs in bacterial DNA-treated *Tlr9*$^{+/-}$ mice and a concomitant decrease in double negative (DN, or CD4^{-}CD8^{-}) T cells. In the kidney, bacterial DNA treatment also decreased the percentage of CD45^{+} cells in *Tlr9*$^{+/-}$ mice. Importantly, all bacterial DNA- induced changes observed in *Tlr9*$^{+/-}$ MRL/*lpr* mice, including those of proteinuria, organ weight, anti-dsDNA IgM, splenic Bregs, splenic DN T cells and renal CD45^{+} cells, were abrogated in *Tlr9*$^{-/-}$ MRL/*lpr* mice. Bacterial DNA treatment of cocultures of B and non-B cells from *Tlr9*$^{+/+}$ and *Tlr9*$^{-/-}$ MRL/*lpr* mice revealed a TLR9-dependent, but B cell-extrinsic, induction of Bregs mediated by IL-6 produced most likely from myeloid cells. Altogether, our study suggests possible attenuation of SLE by gut bacteria-derived DNA through a B cell-extrinsic, TLR9-dependent mechanism that involves the induction of IL-10^{+} Bregs and secretion of potentially protective anti- dsDNA IgM.

SLE Genetics

1401 A GENOME WIDE ASSOCIATION SCAN OF SLE GENETIC RISK IN A COHORT OF AFRICAN-AMERICAN PERSONS

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Systemic lupus erythematosus (SLE) is the prototypical multi-system autoimmune disease with diverse clinical features in persons with disease. SLE is also unified by characteristic auto-immunity directed against nucleic acid or nucleoprotein complexes. SLE is both more prevalent and typically exhibits a more severe clinical course in persons with African-American ancestry than in persons with European ancestry. The reasons for this discrepancy remain incompletely understood. GWAS studies of SLE in cohorts of individuals with Amerindian, East Asian and European ancestry have identified > 180 risk loci for SLE across the genome. These loci act in several pathways: clearance of autoantigens, innate immune response to nucleic acids, and lymphocyte activation. Despite these advances in understanding the genetic basis of SLE, a genome-wide association scan (GWAS) of SLE in a cohort of individuals with African-American ancestry has not yet been reported. Here, we report preliminary results of GWAS in 1494 SLE cases and 6076 matched controls with African-American ancestry. Illumina Infinium Omni 1, Omni 1S, Omni 2.5 and OmniExpress platforms were used for genotyping. Genotypes were imputed using the TOPMed reference panel at NHLBI. By defining the contribution common genetic variants to disease risk across the genome, our study represents a step towards understanding the genetic basis of SLE and the increased prevalence and severity of SLE in African ancestry populations. To fully define the relative contribution of environment and genetics to the discrepant SLE severity and prevalence observed in African-American populations, future studies will be necessary. These studies should focus both on comprehensive understanding of the environmental influences and comprehensive assessment of genome- wide genetic variation (i.e. whole-genome sequencing) that impact SLE risk and disease severity.

Our results confirm genome-wide significant ($P < 5E-8$) association with loci ascertained in other populations (*STAT4-STAT1*, *TNIP1*, *MIR146A*, *HLA-C4A-C4B*, *IRF5*, *BLK*, *PLAT-IKBKB*, *RELA-RNASEH2C-OVOL1*, *ITGAM,* and *IRF8*) and identify several novel genome-wide significant risk loci that are newly described in our study (*ENSA*, *IKBKB/Chr8: Centromere* and *PCMTD1-ST18*). Further, we compared associated variants in our study with those from three large SLE GWAS studies in cohorts of individuals with European, East Asian and Amerindian ancestry. This comparison of SLE risk loci revealed pervasive sharing of SLE genetic risk across ancestral groups. For 70% of the risk loci, the lead marker exhibited nominal association ($P < 0.05$) with SLE in our GWAS of SLE in African- American persons. Importantly, the association of all such variants cohered with the reported direction in other ancestries.

Overall, our findings are consistent with a polygenic contribution to SLE in African-American individuals that is largely shared across populations. We also find association with a rare variant (MAF < 1%) of large effect (OR = 3.91) near a locus previously identified via ImmunoChip (Illumina), *PLAT-IKBKB*. The lead variant at this locus is non-polymorphic in populations with ancestry outside of Africa. This association explains the increased risk of SLE in ~4% of cases in our cohort. On the one hand, our GWAS of SLE in persons African-American ancestry provides insights that reinforce the conclusions concerning the known risk loci from other ancestries. On the other hand, this mechanism uniquely raises SLE risk in a small proportion of African ancestry individuals with SLE. Together our findings reveal both uniformity and diversity of genetic risk factors impacting SLE development across populations.

For Poster Presentation

1501 ENPATORAN: PRECLINICAL EVIDENCE SUPPORTING GLUCOCORTICOID DOSE REDUCTION AND PHASE II STUDY DESIGN IN PATIENTS WITH SLE AND/OR CLE (WILLOW)

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Purpose Enpatoran is a potent selective dual inhibitor of toll-like receptor (TLR) 7 and TLR8. Previous studies have shown aberrant activation of TLR7/8 may be involved in systemic lupus erythematosus (SLE) pathogenesis and glucocorticoid resistance. To assess whether enpatoran could be used in SLE management to avoid the detrimental effects of long-term corticosteroid use, we evaluated its glucocorticoid-sparing effect and designed a basket trial to assess its efficacy and safety in patients with SLE and/or cutaneous lupus erythematosus (CLE).

Methods Cytokine concentrations and gene expression changes were measured in stimulated human peripheral blood mononuclear cells (PBMCs) from healthy donors after treatment with dexamethasone, TLR7/8 inhibitor, or both. A Phase II basket design, proof-of-concept, dose-finding study in patients with SLE and/or CLE (WILLOW) was designed.

Results In healthy donor PBMCs, synergy was observed between TLR7/8 inhibitor and dexamethasone. Combination treatment inhibited cytokine release (interleukin-6) with greater potency than either treatment alone and reduced the expression of nuclear factor-kappa B and interferon-regulated genes. WILLOW is a Phase II, basket proof-of-concept, dose-finding, randomized, double-blind, placebo-controlled 24-week study with two cohorts (NCT05162586, figure 1). The primary objectives of WILLOW are to evaluate the dose–response relationship of enpatoran in reducing disease activity based on Cutaneous Lupus Erythematosus Disease Area and Severity Index-A (CLASI-A) or BILAG- Based Composite Lupus Assessment (BICLA) response rate. The secondary objectives are to investigate effects on both BICLA response and clinically meaningful corticosteroid reduction and evaluate disease control (including clinically meaningful corticosteroid reduction) in patients with predominantly active CLE or SLE. Cohort A will enroll patients with CLE (active subacute CLE and/or discoid LE) or SLE with predominantly active lupus rash. Cohort B, in two parts, will enroll SLE patients with moderate-to-severe systemic disease activity. Part 1 will assess clinical signal

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