

as compared to controls. A combination of inhibitors and gene expression studies showed that glucose oxidation rather than aerobic glycolysis is a major metabolic pathway of lupus T cells. *In vitro*, both metformin and 2-DG blocked IFN γ production and metformin increased IL-2 production. *In vivo*, a combined treatment with metformin and 2-DG normalised T cell metabolism and reversed disease phenotypes in all four lupus mouse models. Remarkably, the number of T_{FH} cells, which correlates with disease severity in patients, was normalised by the combination treatment in every model. Further, excessive IFN γ production by CD4 T cells from SLE patients was also normalised by metformin. Finally, CD4⁺ T cells from lupus patients showed a metabolic signature with over-expression of genes promoting glucose utilisation, mitochondrial oxidative phosphorylation and sterol synthesis.

Conclusions The combination of a glucose inhibitor with metformin restores T cell function and reverses disease across diverse mouse models of SLE, and metformin treatment normalises the function of T cells from SLE patients. We propose that T cell metabolism may serve as a biomarker of disease activity and provides a novel target for immune intervention in SLE.

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AI-26 INTestinal IGA AS PATHFINDERS TO IDENTIFY MICROBIOME PATHOBIONT CANDIDATES IN SLE

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Background SLE is an archetypical systemic autoimmune disease, which has been attributed to interactions between genetic and environmental factors that are currently not well understood. Yet recent reports have begun to elucidate how intestinal bacteria influence the development of physiologic B-cell/T-cell responses, and can affect the pathogenesis of inflammatory and autoimmune conditions. Our studies are designed to shed light on the potential roles of the gut microbiome in SLE pathogenesis.

Methods We have assembled and characterised a cohort of 60 female SLE patients and 20 healthy controls. DNA from the unfractionated bacteria in faecal samples, and from the sorted endogenous IgA-coated and non-coated bacterial fractions, was then extracted. 16 S bacterial rRNA genes were then barcoded and amplified, and over 20,000 reads were determined per sample using illumina NGS technology. Faecal and serum total Ig and autoantibodies were measured by ELISA.

Results Our analysis showed less microbiome diversity in SLE than healthy controls ($p = 0.002$). This dysbiosis was treatment independent, with more severe intestinal dysbiosis and decreased bacterial diversity in patients with high disease activity, based on SLEDAI. In addition, SLE patients had increased representation of certain bacterial families, genus's and species, based on 16 S rRNA assignments of operational taxonomic units (OTUs). Patients with active disease displayed contractions of bacterial taxa with reported protective properties and reciprocal expansions of taxa with putative pathobiont properties. We also assessed IgA, which is the most prevalent antibody isotype made by the human body, and found evidence of exuberant levels in both intestinal and blood samples of SLE patients. While only a minority of bacterial taxa are specifically coated by endogenous intestinal IgA, IgA-coated bacteria in SLE patients had differential

representation with recurrent taxa-specific expansion in SLE patients. Strikingly, *Prevotella copri*, which has recently been linked to new-onset RA, was significantly over-represented among the IgA-coated taxa only in SLE patients with high disease activity, and was not detected in healthy controls.

Conclusion Our studies provide the first evidence that SLE is associated with gut microbiome dysbiosis with expansions of specific bacterial taxa that may contribute to immune dysregulation. This imbalance was more significant in patients with high disease activity. Characterisation of *in vivo* IgA-coated bacteria demonstrated that certain microbes taxa/species are preferentially recognised by the adaptive immune system of SLE patients, and these differ significantly from healthy adults. We are now studying these candidate pathobionts in longitudinal studies to address whether the microbiome predicts and/or tracks flares.

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AI-27 BACTERIAL AMYLOIDS FROM BIOFILMS BREAK TOLERANCE IN LUPUS

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Background Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease, in which infections are considered to play a pathogenic role, but the cellular and molecular culprits remain unknown. Bacterial biofilms are multicellular bacterial communities important in the establishment of chronic infection by pathogens. Bacteria produce amyloids, complex proteins with a conserved beta sheet structure, that strengthen the extracellular matrix of their biofilms. We have reported that bacterial and eukaryotic DNA is incorporated into curli fibres, functional bacterial amyloids present in *Salmonella* and *E. coli* biofilms. We found that curli/DNA complexes from biofilms activate conventional dendritic cells leading to production of pro-inflammatory cytokines, including Type I interferons, *in vitro* and *in vivo*, indicating that curli are a new class of danger signals. I.p. infections with curli-expressing *S. Typhimurium* or *E. Coli* Nissle, and also systemic administration of curli/DNA complexes purified from *Salmonella* biofilms accelerated onset in lupus-prone NZBxW/F1 mice and triggered autoantibodies production in non-predisposed mice, suggesting curli/DNA complexes as novel players in SLE pathogenesis.

Materials and methods In order to determine the cellular and molecular players in the acceleration of lupus by curli-expressing infections, bone marrow-derived cultures of conventional dendritic cells and macrophages were generated *in vitro* and B cells were isolated *ex vivo* from NZBxW/F1 lupus-prone mice, TLR-deficient mice and wild-type mice and stimulated with curli/DNA complexes purified from *Salmonella* biofilms. Moreover, lupus prone and control mice were infected i.p. and by oral gavage with curli-expressing or curli-deficient *S. Typhimurium* and the effects on dendritic cells, macrophages, and B cell activation, and production of autoantibodies were studied. A novel ELISA was developed to measure anti-curli antibodies in SLE patients and infected mice.

Results We found that Curli and DNA synergistically activate innate and adaptive immune cells, *in vitro* and *in vivo*, via TLR2 and TLR9, leading to a Type I interferon response. We found that curli/DNA complexes can directly stimulate proliferation and

activation of B cells and induce autoantibody production. Natural infections with curli-expressing *S. Typhimurium* triggered autoantibodies production in NZBxW/F1 mice and control mice, suggesting curli/DNA complexes break tolerance in SLE. Finally, sera from lupus patients during flares showed elevated levels of anti-curli antibodies.

Conclusions Biofilm-derived curli/DNA complexes are potent activators of innate and adaptive immune cells and can mediate the acceleration of lupus by infections. These studies may provide a novel biomarker of flare and suggest targeting biofilms and bacterial infections as new therapeutic tools in lupus.

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AI-28

HLA-DR3RESTRICTED RESPONSES TO SMD, A LUPUS-RELATED ANTIGEN PROVIDE INSIGHTS TO THE ORIGIN OF LUPUS-RELATED AUTOANTIBODIES AND THE UNIQUE FEATURES OF THE TARGETED ANTIGENS

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Background The presence of complex autoantibodies (auto-Abs) is a hallmark of SLE. The most cited hypotheses for their origin are the “B cell epitope mimicry hypothesis between SmB and EBV” and the “particle hypothesis”. Neither is adequate to explain the characteristics of SLE-related Abs. Our studies showed that HLA-DR3 and DR2 transgenic mice in contrast to DR4 respond well to SmD and Ro60 with DR3 mice being the best responders. In addition, certain bacterial mimics of SmD and Ro60 T cell epitopes were shown to induce auto-Abs to SnRNP and Ro60/La.

Materials and methods T cell epitope mapping was done by the generation and characterisation of T-T hybridomas to SmD and Ro60 and their 15 mers. Antibody specificities to SLE-related antigens (Ags) and their peptides were done by ELISA.

Results At least seven SmD core T cell epitopes were identified. They have no sequence homology. 18 of reactive T-T hybridomas used multiple TCR α and TCR β . Bioinformatics analysis identified more than 10000 potential bacterial mimic peptides with many from commensal bacteria. The binding affinities of the SmD T epitopes were in the medium range among all the relevant mimics. Selected mimic peptides showed that only those with medium binding affinities stimulated related T-T hybridomas to the SmD peptides and only they were able to stimulate Ab responses in patterns similar to that induced by the related SmD peptide. A significant number of T-T hybridomas were reactive with multiple T cell epitopes within the SmD molecules. Some of these hybridomas also were reactive with SmD, SmB and/or A-protein within the snRNP particles and Ro60. In addition, the bacterial T cell mimic peptides often shared B cell epitopes with the related SmD peptide. Also healthy DR3⁺ blood donors had significantly higher Ab titers against SmD. Similar results were obtained in the Ro60/La system.

Conclusions Autoreactive TCRs are part of the normal repertoire that are positively selected for host defense against microbial agents. The presence of multiple intra- and inter-molecular T and B cell epitopes are characteristic of SLE-related auto-Ags.

These characteristics provide a scenario for the inevitability of the presence of auto-Ab and autoreactive-T cells in healthy individuals with susceptible HLA-D regions and provide a mechanism for B cell epitope spreading in SLE. SLE-related Abs are the results of our reaction to exposure to commensal and/or pathogenic microbes. Innate immunity plays an amplifying role. These observations provide the rationale to target microbiome and both adaptive and innate immunities in the treatment of SLE.

AI-29

METABOLOME CHECKPOINTS OF MTOR ACTIVATION AND CLINICAL RESPONSIVENESS IN SLE

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Background The mechanistic target of rapamycin (mTOR) serves as a metabolic sensor of genetic and environmental cues that effectively regulates physiological T-cell activation and lineage specification. mTOR complex 1 (mTORC1) promotes pro-inflammatory T-cell development, B-cell activation and production of antinuclear autoantibodies (ANA) both in patients and mice with systemic lupus erythematosus (SLE). Therefore, we initiated a prospective clinical trials with rapamycin and N-acetylcysteine (NAC), the latter of which blocks redox-dependent mTORC1 activation.

Materials and methods 36 SLE patients were enrolled in a 3-month placebo-controlled trial with NAC which involved 212 metabolic and immunological markers prior to enrollment, and follow-up at 1-month intervals. 42 healthy controls, matched at each visit for age, ethnicity, and gender, were studied in parallel. 258 metabolites were measured mass spectroscopy. 40 SLE patients were enrolled in a 12-month open-label intervention with rapamycin; patients and 88 matched controls were studied in parallel at 3-month intervals. Analysis of pathways, area under the curve (AUC) logistic regression, two-factor (NAC versus placebo) time series within individual subjects were performed with Metaboanalyst. SLEDAI, BILAG, and SRI disease activity indices were calculated.

Results Rapamycin reduced disease activity in 126 ± 18 days as evidenced by well-tolerated rapamycin plasma levels of 8.7 ± 1.2 ng/ml, which was within the targeted therapeutic range of 6–15 ng/ml. SLEDAI disease activity scores were reduced to 5.7 ± 1.0 from 11.8 ± 1.1 at baseline ($p = 0.0028$). Among the patients who completed the 1 year intervention, a SLE Responder Index of 64.3% was achieved. Rapamycin inhibited the pro-inflammatory T cell skewing (Figure 1), including the expansion and IL-4 production of CD4⁺CD8⁻ double-negative (DN) T cells and reversed the contraction of CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs) and CD4⁺ and CD8⁺ central and effector memory T cells. Treatment with NAC exerted lesser but statistically significant reduction of SLEDAI and BILAG over 3-month. Metabolome changes involved 27 of 80 KEGG pathways at FDR $p < 0.05$ with most prominent impact on the pentose phosphate pathway (PPP). While cysteine was depleted, a PPP-regulated compound, kynurenine, was the most increased metabolite and the top predictor of SLE (AUC = 0.859). Kynurenine directly stimulated mTORC1 activity of DN T cells *in vitro*. Relative to placebo, NAC reversed these metabolite changes *in vivo*.

Conclusions The PPP-connected accumulation of kynurenine and its stimulation of mTORC1 are identified as metabolic