Conversely, regression analyses demonstrated that decreased tricarboxylic acid (TCA) cycle and lipid metabolism signatures reflected decreased kidney cell transcripts, which was confirmed by the negative relationship between the TCA cycle signature and expression of the tubule damage marker, *HAVCR1*.[1] Indeed, similar alterations to metabolism were observed in some less severe LN patients, in which inflammatory cell transcripts were not yet increased.[2] Although interferon can induce metabolic changes at the transcriptional level,[3] examination of metabolic gene expression in the murine interferon alpha-accelerated LN model revealed that metabolic changes were not driven by acute exposure to type I interferon.[2] Moreover, evaluation of metabolic changes in murine LN following various treatments demonstrated that metabolic gene expression could be restored with immunosuppressive therapy.[2] Regression analyses in DLE and PSO demonstrated that there was a positive correlation between the glycolysis signature and the keratinocyte signature, whereas in PSO, AD, and SSc the glycolysis signature was positively correlated with the monocyte/myeloid cell signature.

Conclusion These results indicate that altered metabolic dysfunction is a common change in lupus- and inflammatory disease-affected tissues and appears to reflect immunologic damage.

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400 – Innate immunity/cardiovascular disease and lupus

401 TRIM21 AS A REGULATOR OF UVB-DRIVEN IFN RESPONSES IN LUPUS

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Background Patients with systemic lupus erythematosus (SLE) experience photosensitivity, with exposure to ultraviolet light (UV) driving lupus flares, triggering symptoms like joint pain and fatigue, in addition to causing cutaneous lesions. Our previous work has shown that TRIM21, an autoantigen in SLE, functions as a negative regulator on the pathways driving IFN expression. *Trim21*$^{-/-}$ mice develop systemic autoimmunity due to an inability to regulate type I IFNs. These mice also spontaneously develop skin lesions, leading us to hypothesize that *Trim21*$^{-/-}$ mice may have enhanced sensitivity to UV-induced skin inflammation.

Methods Wild type (WT, C57BL/6) and TRIM21 KO mice were irradiated with UVB (100 mJ/cm^2) consecutively for 1 and 3 weeks, and blood, spleen and kidney analyzed by qPCR, histology, and flow cytometry. Bone marrow derived macrophages (BMDMs) and mouse skin fibroblasts (MDF) were analyzed by qPCR and western blotting. Peripheral blood samples from SLE patients were analyzed by qPCR for interferon stimulated genes (ISG) RSAD2, IFI44, IFI44L, IFI27 and used to calculate IFN scores.

Results Macroscopically, both WT and *Trim21*$^{-/-}$ mice developed similar levels of erythema, edema and induction of inflammatory cytokines in response to UVB exposure. However, compared to WT mice, expression of IFNβ and ISGs were elevated in the skin of *Trim21*$^{-/-}$ mice following UVB. Most notably after UVB exposure, we observed splenomegaly and enhanced expression of ISGs in the blood and spleen of *Trim21*$^{-/-}$ mice. *Trim21*$^{-/-}$ mice also demonstrated enhanced total IgG levels in serum, significantly increased deposition of IgG in the skin and increased ISG expression in the kidneys, all suggesting that loss of TRIM21 in mice results in enhanced IFN-driven responses systemically. Enhanced basal, UVB- and cGAMP-dependent IFNβ expression was observed in *Trim21*$^{-/-}$ BMDMs and MDFs which was restored in BMDMs from *Trim21*$^{-/-}$/Sting$^{-/-}$, suggesting the cGAS-STING pathway was inappropriately regulated in these absence of TRIM21. As TRIM21 is an E3 ligase and known to regulate protein stability, we assessed levels of cGAS, DDX41 (a DNA sensor and regulator of STING signaling) and STING itself. Mechanistically, we found only DDX41 protein levels were stabilized in *Trim21*$^{-/-}$ MDFs or BMDMs, respectively, suggesting it to be a target on this pathway.

Conclusions Taken together our results indicate that TRIM21 protects against IFN induction both at a local and systemic level in response to DNA sensing. Understanding the role of TRIM21 in preventing systemic disease will have important understandings of its role in driving increased disease activity and flare in SLE.

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402 GENETIC DISSECTION OF TLR9 REVEALS COMPLEX REGULATORY AND CRYPTIC PRO-INFLAMMATORY ROLES

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Background Toll like receptors (TLR) 7 and 9, endosomal sensors for ssRNA and dsDNA, are key mediators of lupus autoreactivity. Although considered homologous, they have opposing effects on lupus severity: TLR7 exacerbates disease while TLR9 protects. One theory is that TLR9 induces protection by competing with TLR7 for endosomal trafficking, thus restraining TLR7 expression and signaling. However, since both TLR9 and TLR7 ligands are available in lupus, and both TLRs are supposed to signal via MyD88, this theory doesn't explain the differential effects of the two TLRs on disease. To reconcile this, we hypothesized that TLR9 could induce counter-regulatory signaling and/or regulate TLR7 via a ligand- or signaling-independent mechanism.

Methods and Results To differentiate between these hypotheses, we created mutants of TLR9 that prevent ligand binding (TLR9^{K51E}) or MyD88 signaling (TLR9^{P915H}) in the TLR9 locus of MRL/lpr mice. Mutant mice were assessed by for dermatitis, nephritis, immune dysregulation, and auto-antibodies.

TLR9$^{K51E/K51E}$ mice had increased survival and decreased kidney pathology compared to TLR9$^{-/-}$ mice, indicating that

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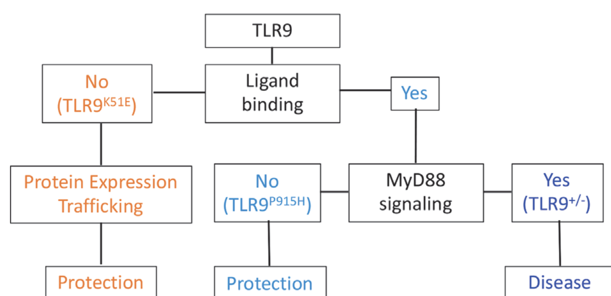

Abstract 402 Figure 1 Model explaining the implications of the TLR9 mutant disease phenotypes

the restoration of TLR9 expression induces protection. To assess the effect of TLR9-MyD88 signaling, we compared $TLR9^{P915H/P915H}$ and TLR9-sufficient cohorts of MRL/lpr mice. Kidney disease, survival and immune activation were significantly more severe in $TLR9^{\pm}$ mice. Thus, there is a TLR9-MyD88 dependent pathway that promotes disease. Moreover, $TLR9^{K51E/-}$ mice had increased glomerulonephritis and immune activation compared to $TLR9^{P915H/P915H}$ mice, suggesting that TLR9 could regulate disease through a ligand binding-dependent but MyD88-independent mechanism (figure 1).

Using a 3 way ($TLR9^{WT}$, $TLR9^{P915H}$ and $TLR9^{-/-}$)-mixed bone marrow chimera, we found that TLR9 inhibits B cell development and differentiation in a B cell-intrinsic fashion and that the absence of TLR9 ($TLR9^{-/-}$) was very different from the inability of TLR9 to signal ($TLR9^{P915H}$). RNA seq analysis of sorted age-associated B cells (ABC) revealed that $TLR9^{WT}$, $TLR9^{P915H}$ and $TLR9^{-/-}$ ABC exhibit different transcriptional programs. Notably, the absence of TLR9 did not lead to an increase in genes that are induced by TLR7, arguing against the idea that TLR9 simply restrains TLR7 signaling.

Conclusion This in vivo genetic dissection of TLR9 reveals how it both promotes and regulates lupus. Inability of TLR9 to signal via MyD88 is different from absence of TLR9 and also is different from inability of TLR9 to bind ligand. These findings shed light on the basic biology of endosomal TLR signaling and are relevant to the design of TLR-targeted therapy.

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403 BACTERIAL BIOFILM PRODUCT CURLI/EDNA INDUCES NEUTROPHIL EXTRACELLULAR TRAPS AND SERUM ANTI-CURLI/EDNA LEVELS CORRELATE WITH BACTERIURIA AND LUPUS ACTIVITY

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Background Infections are a major contributor to lupus disease. We have previously demonstrated that bacterial amyloid curli, produced by E.coli, can accelerate disease in mouse models of lupus. Interestingly curli incorporates extracellular DNA, which in turn can be both adjuvant and a self-antigen in lupus. Uropathogenic E. coli (UPEC) is responsible for the majority of urinary tract infections in SLE. Based on our previous results, we hypothesize that exposure to UPEC triggers anti-curli/eDNA antibodies and curli/eDNA complexes can trigger the innate immune system.

Methods We investigated 98 lupus patients who met at least 4 SLICC criteria. Results were compared to 54 age, sex and race matched healthy controls. We tested the production of anti-curli/DNA complex for both IgG and IgA subclasses. We than correlated the levels of anti-curli/DNA antibodies with clinical parameters. Finally, we treated human neutrophils with curli/eDNA complexes.

Results We found that curli/eDNA induces neutrophil extracellular traps in a ROS-dependent manner. Anti-curli/eDNA IgG levels were detected in lupus and controls plasma and the levels correlated with persistent bacteriuria ($p<0.05$) and disease flares in lupus patients. In addition, anti-dsDNA could bind to anti-curli/eDNA complexes.

Conclusions We conclude curli/eDNA complexes can activate the innate and adaptive immune system and could be a mechanism to sustain disease in lupus.

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404 PLATELETS ARE A SOURCE OF EXTRACELLULAR MITOCHONDRIA AND MITOCHONDRIAL DNA IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background The accumulation of DNA and nuclear components in blood and their recognition by autoantibodies play a central role in the pathophysiology of systemic lupus erythematosus (SLE). Despite the efforts, the sources of these circulating autoantigens in SLE are still unclear. While damaged organs and activated cells are generally considered as potential sources of autoantigens, platelets are often overlooked given that they are anucleated and thereby cannot release genomic DNA. However, accumulating findings suggest that mitochondria are also targeted by antibodies in SLE.

Methods We examined the presence of extracellular mitochondria in blood of patients with SLE and determined correlations with platelet activation. Because mice lack FcγRIIA and murine platelets are completely devoid of receptor capable of binding IgG-containing immune complexes, we generated transgenic lupus mice expressing FcγRIIA for our in vivo investigations. We used a reporter mouse with red fluorescent protein targeted to the mitochondrion to identify the cellular source of the extracellular mitochondria.

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