Results Lymphoid hypertrophy, autoantibody production, serum cytokine levels, and other indicators of immune activation were markedly increased in STING$^{-/-}$ autoimmune-prone mice compared to STING$^{+/+}$ littermates. As a result, STING$^{-/-}$ autoimmune-prone mice had significantly shorter lifespans than controls. TLR-dependent systemic inflammation during TMPD-mediated peritonitis was similarly aggravated in STING$^{-/-}$ and cGAS$^{-/-}$ mice. Mechanistically, cGAS and STING-deficient macrophages failed to express negative regulators of immune activation, and thus were hyper-responsive to TLR ligands. This hyper-reactivity corresponds to dramatically elevated numbers of inflammatory macrophages and granulocytes *in vivo*.

Conclusions Our findings reveal an unexpected negative regulatory role for STING during chronic inflammation. While the dysregulation of TLR7/9 signalling is a recurrent theme in systemic autoimmune, numerous studies have now revealed a *protective role* for TLR9 in SLE. Importantly, the exacerbated disease we observed in STING/lpr mice resembles that reported for TLR9/lpr mice and implies common protective mechanisms originating from STING and TLR9. Although the precise mechanism remains an open question, it is clear that cGAS/STING-dependent pathways maintain a threshold of negative regulators. We propose a similar setting of thresholds from TLR-dependent pathways and further suggest that such coordinated induction of cell-intrinsic thresholds of negative regulators is key in offsetting inflammation. Our data raise a cautionary note regarding the use of newly developed STING-directed therapeutics in systemic disease, because they may have unintended consequences and perturb a carefully orchestrated balance between cytosolic and endosomal signalling cascades.

II-14 DISTURBED CLEARANCE OF APOPTOTIC DEBRIS IN PRISTANE-TREATED TLR9KO MICE LEADS TO ACCUMULATION OF A UNIQUE MACROPHAGE POPULATION

Kerstin Nundel*, Anette Christ, Shruti Sharma, Ann Marshak-Rothstein. *University of Massachusetts Medical School, Department of Medicine, Worcester, USA*

10.1136/lupus-2016-000179.44

Background Nucleic acid binding TLRs have been found to play a critical role in the production of autoantibodies and disease development in animal models of SLE. Intriguingly, TLR9 appears to play both a protective and disease promoting role. While TLR9 is required for the production of anti-dsDNA autoantibodies, TLR9^{KO} autoimmune-prone mice develop more severe disease than their TLR9-sufficient counterparts. Studies from our group and others have pointed to B cell expression of TLR9 as a key determining factor. However, our recent studies point to an additional role for TLR9in myeloid lineage cells.

Materials and methods Pristane injected BALB/c wildtype (WT) and TLR9^{KO} mice were analysed for disease severity at 5 months. Kidney sections were stained for IgG deposition and Ly6G positive cells. Single cell suspensions of different tissues were analysed using flow cytometry. Mixed BM chimaeras (50% WT: 50% TLR9^{KO}) were injected with pristane and myeloid populations were analysed. For apoptotic cell clearance, WT and TLR9^{KO} bone marrow derived macrophages (BMDM) were stimulated with CFSE labelled apoptotic cells and analysed 24 hours later by confocal microscopy.

Results We have found that TLR9-deficiency dramatically exacerbates the onset of renal disease resulting in decreased survival. Increased levels of IgG accumulate in pristane treated TLR9^{KO} glomeruli compared to WT glomeruli, and the increased IgG deposits are associated with an increased myeloid infiltrate. Moreover, this myeloid infiltrate contained an increased frequency of granulocytes a well as an unusual CD11b^{+} Ly6C^{int} Ly6G^{int} (Ly6CG^{int}) subset. To better understand the origin of these populations, the myeloid subsets of pristane-treated mixed (TLR9^{WT} + TLR9^{KO}) BM chimaeras were analysed. Remarkably, the Ly6CG^{int} population was entirely derived from the TLR9^{KO} stem cells. Morphologic analysis revealed that the Ly6CG^{int} population are macrophages containing large lipid droplets, suggesting a role for TLR9 in degradation of pristane. Further *in vitro* analysis of BMDMs stimulated with apoptotic cells showed that most WT BMDMs cleared apoptotic cells by 24 h. However, a large fraction of TLR9-deficient BMDMs still had un-degraded apoptotic cells in the lysosomal compartment, suggesting a role for TLR9 in clearance.

Conclusions These data demonstrate a direct effect of TLR9-deficiency on the expansion of a unique CD11b^{+} population, and further suggest that these cells play a major and direct role in the accelerated disease characteristic in TLR9^{KO} mice. Furthermore, a specific role for TLR9 in the clearance of apoptotic cells may be the underlying cause for the accumulation of this CD11b + subset.

II-15 INHIBITION OF TLR RECOGNITION OF SELF NUCLEIC ACIDS BY PLASMACYTOID DENDRITIC CELLS USING OLIGONUCLEOTIDE-BASED INHIBITORS IN LUPUS

Franck J Barrat*. *The Hospital for Special Surgery, Auto immunity and Inflammation Program, 535 E.70th St., New York*

10.1136/lupus-2016-000179.45

Background SLE is an autoimmune disease where the immune tolerance to self-nucleic acids is broken with devastating consequences. The hallmark of the disease is an increased IFN-α signature in the blood which is accompanied with high levels of autoantibodies and disease activity. Self-nucleic acid recognition by Toll-like receptors (TLR)7 and TLR9 on B cells and plasmacytoid dendritic cells (PDC) is believed to be key in the pathogenesis of SLE promoting immune complexes (IC) and the production of type I IFN, both of which are associated with the severity of the disease.

Results We have generated and described oligonucleotide-based bi-functional inhibitors of TLR7&9 (called ImmunoRegulatory Sequences, IRS) and have shown that these can block IFN production by PDC as well as B cell activation. In addition, IRS are active in vivo and treatment of lupus-prone mice lead to reduced disease symptoms and end-organ damage. SLE patient are often treated with glucocorticoids (GC) but under maintenance levels often suffer from disease flares that necessitate high dose pulse therapy. We have shown that PDC were significantly more resistant to GC induced death in lupus-prone mice, a phenomenon that was completely reversed by pre-treatment with TLR7&9 inhibitor. These data provide a new understanding of the role of self-recognition of DNA and RNA by TLR as an important parameter during inflammatory response. These data also stress the potential utilisation of TLR7&9 specific inhibitors as corticosparing drugs which would be open new possibilities with respect

to therapeutic applications. Finally, we have shown that IRS can prevent skin lesions following mechanical injury by blocking PDC activation in the skin environment. The lead IRS inhibitor, called DV1179, has entered a human clinical trial and its safety was assessed in multiple ascending doses in healthy volunteers and lupus patients, however no effect on IFN response was observed in lupus patients.

Conclusions These data provide a new understanding of the role of self-recognition of DNA and RNA by TLR as an important parameter during inflammatory response. These data also stress the potential of blocking PDC activation to increase patient's response to corticosteroid treatment although the use of oligonucleotide based inhibitors did not reduce the IFN signature in lupus patients. Other approaches could be tested as well.

II-16 PROTECTION OF LUPUS NEPHRITIS BY IRHOM2 DEFICIENCY IN *FCRγIIB-/-* MICE

[1]Xiaoping Qing, [1]Yuri Chinenov, [2]Michael Madaio, [1]Patricia Redecha, [3]Priya D Issuree, [4]David McIlwain, [5]Tak W Mak, [6]Carl P Blobel, [1,7]**Jane E Salmon***. *[1]Program in Inflammation and Autoimmunity, Hospital for Special Surgery, USA; [2]Department of Medicine, Medical College of Georgia at Augusta University, USA; [3]Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, USA; [4]Department of Microbiology and Immunology, Stanford University, USA; [5]Princess Margaret Cancer Centre, University Health Network, Canada; [6]Department of Medicine, Department of Physiology, Systems Biology and Biophysics, Weill Medical College of Cornell University, USA; [7]Department of Medicine, Weill Medical College of Cornell University, USA*

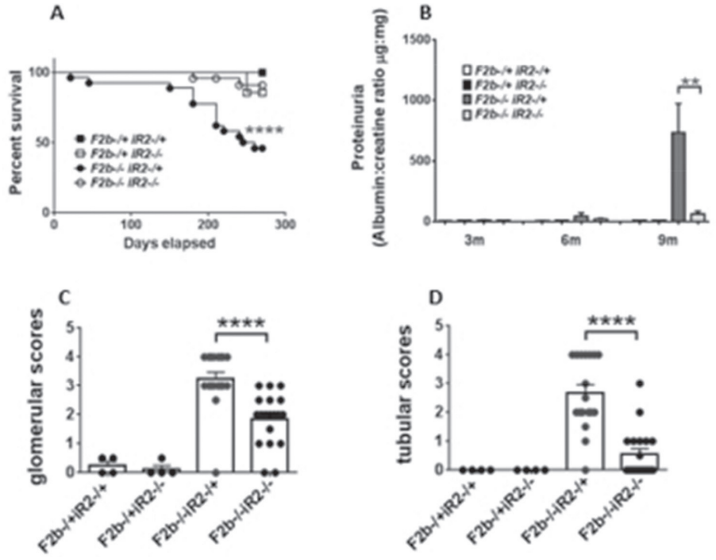
10.1136/lupus-2016-000179.46

Background Lupus nephritis (LN) is a major cause of morbidity and mortality in lupus. A disintegrin and metalloprotease 17 (ADAM17), is a principal membrane-anchored metalloprotease that cleaves a large spectrum of membrane-bound proteins into their soluble forms. Inactive rhomboid protein 2 (iRhom2), a newly identified regulator of ADAM17, controls maturation and function of ADAM17. Interestingly, in iRhom2$^{-/-}$ mice, loss of ADAM17-dependent shedding activity is limited to the immune organs. Accumulating evidence has shown increased protein shedding and possibly activation of ADAM17 in lupus. Among ADAM17 substrates, tumour necrosis factor α (TNF-α) and heparin-binding EGF (HB-EGF) have been reported to play important roles in mediating renal damage in lupus. We hypothesised that the activation of iRhom2/ADAM17 pathway plays a role in the pathogenesis of lupus nephritis.

Materials and methods We crossed *iRhom2*$^{-/-}$ mice with the well-established *FcRγIIB-/-* lupus-prone mice, and assessed development of lupus-like syndrome in these mice.

Results We found that *iRhom2* deficiency protects *FcRγIIB*$^{-/-}$ mice from severe kidney damage (Figure 1), with minimal impact on the production of anti-double stranded (ds) DNA antibodies and renal deposition of immune complex and complement C3. In the absence of iRhom2, glomerular and tubule-interstitial structures were preserved, and massive inflammatory infiltrates including myeloid and CD4$^+$ T cells were alleviated in the lupus kidneys. Protection of kidney injury by *iRhom2* deficiency is associated with reduced EGFR signalling and ERK1/2 activation in the kidneys of *FcRγIIB*$^{-/-}$ mice. Transcriptome analysis of the whole kidneys as well as kidney macrophages from *FcRγIIB*$^{-/-}$ mice identified genes encoding pro-inflammatory cytokine/chemokines, fibrosis and tissue remodelling highly upregulated, and many of these genes were significantly reduced in the absence of *iRhom2*. In addition, kidney biopsies from patients with lupus nephritis show intense staining for HB-EGF, an EGFR ligand, in areas of crescents.

Conclusions Our findings here provide the first evidence that iRhom2, a major regulator of ADAM17, plays a critical role in the pathogenesis of LN. The role of iRhom2 in a spontaneous chronic mouse model of LN, *FcRγIIB*$^{-/-}$ mice, appears to be targeting at the effector arm of the disease, rather than affecting the process of autoimmunity development. iRhom2 may be a potential therapeutic target in LN.



Abstract II16 Figure 1 *iRhom2* deficiency protects *FcγRIIB-/-* mice from developing severe kidney injury. *FcγRIIB-/-* mice crossed with *iRhom2-/-* mice were assessed for survival and kidney injury. Survival **(A)** and proteinuria **(B)** were plotted at each age. Pathological scores were illustrated for glomerular **(C)** and tubular-interstitial area **(D)** respectively. (A) survival, Chi square test. (B) proteinuria, student *t*-test. (C, D) pathological scores, non-parametric Mann Whitney test. ** $P < 0.005$, **** $P < 0.0001$