Acknowledgements Andrew D. Paterson, Genetics and Genome Biology, Research Institute, the Hospital for Sick Children, Toronto, Canada

John Harley, Centre for Autoimmune Genomics and Aetiology, Cincinnati Children's Hospital Medical Centre, Cincinnati, USA

Funding for this work was provided by:
NIH R01 AI070983
NIH K24 AI078004
NIH T32 AR007534
University of Colorado School of Medicine
University of Colorado Department of Medicine
Charley J. Smyth Endowed Chair for Rheumatology Research

GG-12 ALTERED EXPRESSION OF LONG NONCODING RNA IS ASSOCIATED WITH A LUPUS-ASSOCIATED VARIANT IN COMPLEMENT RECEPTOR 2

Brendan M Giles, Bryan T Nycz, **Susan A Boackle***. *Division of Rheumatology, University of Colorado School of Medicine, USA*

10.1136/lupus-2016-000179.64

Background Systemic lupus erythematosus is a multisystemic autoimmune disease characterised by the production of autoantibodies to nuclear antigens. We have identified a variant in intron 1 of complement receptor 2 (*CR2*/*CD21*) that is associated with decreased risk of lupus (rs1876453; $P_{meta} = 4.2 \times 10^{-4}$, OR = 0.85). Its effect is strongest in subjects with anti-dsDNA antibodies (case-control $P_{meta} = 7.6 \times 10^{-7}$, OR = 0.71; case-only $P_{meta} 1.9 \times 10^{-4}$, OR = 0.75), suggesting a preferential association with this endophenotype. rs1876453, located 97 nucleotides from the 5' end of *CR2* intron 1, alters the binding of multiple protein complexes, including one containing CTCF, and is associated with increased B cell-specific expression of the adjacent gene, complement receptor 1 (*CR1*/*CD35*). The transcriptional mechanism connecting these observations remains unclear, and we hypothesised that long noncoding RNA (lncRNA) play a role.

Materials and methods cDNA was generated by reverse transcription from RNA purified from the Raji B cell line as well as from human tonsil and spleen, peripheral blood mononuclear cells, and purified primary B cells. PCR was performed using 5' and 3' primers that targeted spliced exons from known lncRNA sequences in the intergenic region 5' of *CR2,* in the *CR2* gene, and in *CR1* intron 1. Quantitative PCR of primary B cell transcripts was performed using cDNA transcribed using random primers and MultiScribe reverse transcriptase (Applied Biosystems), customised lncRNA primers and probe that targeted spliced exons, Taqman assays for *U6 snRNA* and *b-actin* mRNA, and the Applied Biosystems 7500 Real-Time PCR System. Relative expression levels of lncRNA, normalised to either *U6 snRNA* (A) or *b-actin* (B), were calculated using the comparative C_T method. P values were determined using a two-tailed Student t test and a p value of <0.05 was considered significant.

Results We confirmed the presence of annotated lncRNAs in the *CR2-CR1* genomic region in various cell types. One annotated lncRNA located downstream of rs1876453 in *CR2* intron 1 was readily detected in B cells. We determined the allele-specific expression of this lncRNA by quantitative RT-PCR and found that it was ~3-fold increase in individuals with the minor protective allele at rs1876453 ($p = 0.0025$ normalised to *U6 snRNA* and $p = 0.0054$ normalised to *beta-actin*).

Conclusions Our data suggest that the generation of pathogenic autoantibodies associated with early, active, and severe lupus is modified by expression of a *CR2* lncRNA that appears to have long-range effects. Examination of its mechanism and effects may therefore reveal a novel target for the treatment of lupus.

Acknowledgements This study was approved by the Colorado Multiple Institutional Review Board (Protocol 06–0501).

GG-13 PATHOGENESIS OF LUPUS PROLIFERATIVE GLOMERULONEPHRITIS (LPGN): PODOCYTES AS TARGETS AND RESPONDERS PROVIDE EVIDENCE FOR THE IMPORTANCE OF LOCAL FACTORS CONTROLLING END ORGAN DAMAGE

[1]**Shu Man Fu***, [1]Hongyang Wang, [1]Chao Dai, [1]Sun-sang J Sung, [2]Felicia Gaskin. [1]*Division of Rheumatology and Immunology, the Centre of Inflammation, Immunity and Regenerative Medicine and Division of Nephrology, Department of Medicine, University of Virginia, Charlottesville, VA, USA*; [2]*Department of Psychiatry and Neurobehavioral Sciences University of Virginia, Charlottesville, VA, USA*

10.1136/lupus-2016-000179.65

Background LPGN is the prototypic immune-complex (IC) mediated disease. The current dogma that IC deposition with complement (C') activation inevitably leads to renal damage is neither sufficient nor comprehensive to account for the pathogenesis of LPGN. Our genetic studies support the thesis that acute GN (aGN) with IC deposition with C' activation and cell infiltration and mesangial proliferation is a distinct phenotype from chronic GN (cGN) that is characterised by glomerulosclerosis, tubular dilatation and interstitial fibrosis with severe proteinuria and premature mortality. Furthermore, circulating ANA and anti-dsDNA Abs are not required for LPGN.

Materials and methods Female mice of NZM2328 and its intrachromosomal recombinant congenic line NZM2328.Lc1R27 (R27) were used. Anti-GBM induced GN was used as a model for IC mediated LPGN. Immunofluorescence was used to identify cell populations that made cytokines and complement components.

Results R27 developed aGN and mild proteinuria without progression to cGN, end stage renal failure and early mortality. The kidneys of aged R27 had IC deposition and cellular infiltration, not distinguishable from that of aGN in NZM2328. Multiple approaches showed that the lack of progression from aGN to cGN in R27 was due to podocyte resistance to IC-mediated damage, a phenotype controlled by the allelic *Cgnz1* gene.

With a novel method to study intra-glomerular cytokine production, NZM podocytes were shown to be the major cell population that makes IL-1β in cGN, infiltrating CD11b+ macrophages make TNFα and the mesangial cells make IL-6. R27 mice do not show this compartmentalization of cytokine production. Preliminary data showed that the podocytes in Class III and IV lupus GN make IL-1β.

Podocytes at the early and late cGN were shown to make C1q and C3. The expression of these complement components is less evident in R27 kidneys. C1q and C3 were present in some podocytes in biopsies of class III and IV lupus nephritis. Urinary podocytes making C1q were detected by us in of four patients with LPGN but not in normal individuals.

Conclusions Our studies of lupus GN in both mouse and in man have provided significant information and insight regarding the role of podocytes as targets and as responders to IC mediated injuries. Our results suggest that the pathogenesis of LPGN should be revisited with focus on the local factors that may be of

Lupus Sci Med: first published as 10.1136/lupus-2016-000179.65 on 31 August 2016. Downloaded from http://lupus.bmj.com/ on April 19, 2024 by guest. Protected by copyright.

paramount importance. In addition to enhance our basic knowledge of podocyte biology, our results may provide novel targets for intervention and new urinary biomarkers to monitor therapeutic responses.

Interferons

I-01 NOVEL MECHANISM OF ACTION OF ANTI-MALARIAL DRUGS IN THE INHIBITION OF TYPE I INTERFERON PRODUCTION

Jie An, Joshua Woodward, Mark Minie, Tomikazu Sasaki, **Keith Elkon***. *University of Washington, Department of Medicine and Immunology, Biochemistry, Bioengineering, Medicinal Chemistry, University of Washington, Seattle, WA, USA*

10.1136/lupus-2016-000179.66

Background Anti-malarial drugs (AMD) such as Hydroxychloroquine (HCQ) and Quinacrine (QC) are effective in the treatment of skin rash and arthritis in systemic lupus erythematosus (SLE). However, which mechanism(s) are responsible for their beneficial action is uncertain. Type I interferon, (IFN-I) is strongly implicated in the pathogenesis of SLE and 'interferonopathies' such as Aicardi-Goutieres Syndrome (AGS) A new DNA activated IFN-I pathway, cyclic GMP-AMP (cGAMP) synthase (cGAS), was recently discovered and linked to AGS and mouse models of Lupus. Preliminary data indicate that a subset of SLE patients also have elevated cGAs and cGAMP (the cyclic dinucleotide responsible for activation of STING and IFN-I.
Materials and methods *In silico* structure-based drug screening were provided by the CANDO docking algorithm. Predictions made by CANDO were confirmed by Autodock Vina and analysed via PyMOL. cGAS activity/cGAMP production was analysed by Thin Layer Chromatography (TLC). DNA-binding to cGAS in the presence or absence of AMD was determined by an Electrophoretic Mobility Shift Assay (EMSA). Following DNA cell transfections, cytokines were quantified by qPCR, ELISA or an ISRE-luciferase reporter assay. cGAMP in patient samples was quantified by mass spectrometry.
Results In silico screening of drug libraries identified several antimalarial drugs (AMD) which could potentially inhibit cGAS activity by interacting with cGAS/DNA dimer complex. Electrophoretic Mobility Shift Assay revealed that AMD disrupted the double stranded DNA-cGAS complex in a dose dependent manner. These AMD also inhibited IFN-I expression in THP1 cells transfected with dsDNA and in 293 T cells transfected with cGAS/STING plasmids validating that cGAS is a target of AMD. We synthesisedsynthesized several new AMD. One of these compounds, X6, had excellent water solubility and cell penetration. X6 localised to the cytosol and had a lower toxicity profile compared to QC. Biochemical and cellular assays revealed that X6 was a more potent inhibitor of IFN-I production than HCQ. We also validated mechanism of action and proof of concept in the animal model of AGS.
Conclusions Our studies identify new DNA sensor cGAS as a target of AMD activity, which provide a novel mechanism of action of these AMD. We have synthesised new AMD like drugs that are also able to inhibit cGAS as well as Toll pathways. These drugs could be beneficial for the treatment of AGS and/or Lupus.

I-02 INCREASED INTERFERON β EXPRESSION AND SENESCENCE ASSOCIATE SECRETORY PHENOTYPE IMPAIR THE IMMUNOMODULATORY FUNCTION OF BONE MARROW MESENCHYMAL STROMAL CELLS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Lin Gao, Jennifer Anolik, **Richard Looney***. *Department of Medicine, Division of Allergy, Immunology and Rheumatology, University of Rochester Medical Centre, Rochester, NY 14642*

10.1136/lupus-2016-000179.67

Background Interferon I (IFN-I) signature is an important feature of systemic lupus erythematosus (SLE). Our previous study identified an IFN-I signature in both bone marrow (BM) and peripheral blood of SLE patients. The overlapping roles of IFNα subtypes and disappointing results with IFNα subtype blockade in clinical trials calls for alternative targets and recent findings centred on IFNβ suggest that it is an important candidate molecule in SLE. IFNβ has distinct features as compared to IFNα: higher affinity binding to the shared IFN-I receptors, IFNβ specific gene transcripts, induction of senescence in fibroblast. As a critical non-hematopoietic component in BM, MSCs create a microenvironment for hematopoiesis and immunity. MSCs display robust immunomodulatory properties. MSC defects have been suggested in autoimmune diseases. Taking into consideration the importance of IFNβ and MSCs in autoimmune diseases, here we set out to investigate the role of IFNβ and MSC in SLE pathogenesis, and the underlying mechanisms.
Materials and methods BM MSCs were isolated with FicollPaque gradient centrifugation (1.073 ± 0.001 g/ml) and phenotyped using flow cytometry. Various *in vitro* approaches including confocal immunofluorescence immunocytochemistry, real-time PCR, western blotting, comet assay, beta-galactosidase assay and RNA interference were applied.
Results We compared 6 age paired BM aspirates from healthy controls and SLE patients. SLE MSCs show reduced proliferation rate, increased production of reactive oxygen, and increased DNA damage and repair (DDR), which leads to p53 mediated senescence associate secretory phenotype (SASP) and inhibited immunomodulatory factors production. IFNβ increased 5 folds and IFNβ specific genes are significantly elevated ($p < 0.05$) in SLE BM MSCs and are closely correlated to the level of Mitochondrial Antiviral Signalling Protein (MAVS) ($r > 0.9$, $p < 0.01$), an intracytoplasmic nucleic acid sensor. Silencing MAVs inhibits IFNβ expression and reverses SASP in SLE MSCs.
Conclusions SLE is associated with elevated IFN-I in BM. BM MSCs produce IFNβ, have increased DDR and SASP. Thus an IFNβ positive feedback loop forms in SLE BM MSCs. By silencing MAVS, also named Interferon Beta Promoter Stimulator Protein 1, IFNβ expression is inhibited and IFNβ positive feedback loop is disrupted. Moreover, SASP is rescued by MAVs blockage in SLE BM MSCs. Our novel findings of the IFNβ positive feedback loop and related SASP in SLE BM MSCs shed light on SLE pathogenesis. In addition, our study has also revealed the essential role of MAVS in IFNβ positive loop, and thus provided a new potential therapeutic target for SLE treatment.

Lupus Sci Med: first published as 10.1136/lupus-2016-000179.65 on 31 August 2016. Downloaded from http://lupus.bmj.com/ on April 19, 2024 by guest. Protected by copyright.