AA-02 THE EXPRESSION OF AUTOANTIBODIES TO MITOCHONDRIA IN THE BLOOD OF PATIENTS WITH SLE

[1]David S Pisetsky*, [1]Diane M Spencer, [2]Fariborz Mobarrez, [2]Enrico Fuzzi, [2]Elisabet Svenungsson. *[1]Department of Medicine, Duke University Medical Center; Medical Research Service, Durham VA Medical Center, Durham, North Carolina USA; [2]Unit of Rheumatology, Department of Medicine, Solna, Karolinska Institutet, Karolinska University Hospital, SE-171, 76 Stockholm, Sweden*

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Background Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the expression of antibodies to extracellular vesicles (EVs). These vesicles can arise from dead and dying cells and display nuclear and cytoplasmic molecules. Since EV preparations contain mitochondria, we performed experiments to test directly the binding of SLE antibodies to mitochondria.

Methods Mitochondria were prepared from mouse liver and immobilized on microtiter plates pre-coated with poly-l-lysine at 0.5 µg/ml. Bound antibodies were detected using a peroxidase-conjugated anti-IgG reagent. To determine whether DNA contributed to the antigenicity of the mitochondria, the ability of DNA to inhibit binding was tested. The binding of sera from 211 SLE patients who met 1982 ACR criteria for classification was determined by ELISA and results compared with an ELISA for M2 antimitochondrial antibodies (AMA).

Results Using an ELISA assay with immobilized mitochondria, 60.2% of SLE sera showed positive responses defined as greater than two standard deviations above the mean of control sera. Samples were also analyzed using a commercial AMA ELISA (Euroimmun US, Morris Plains, New Jersey) for IgG antibodies to the M2 antigen. With this kit, 5.7% of the SLE samples tested positive. To determine the relationship of antibodies to anti-DNA, the ability of calf thymus (CT) DNA to inhibit binding to mitochondria was investigated for a subset of samples. In the ELISA, CT DNA at 50 µg/ml inhibited binding for 7 of 8 SLE plasmas, with inhibition ranging from 7.7%–59.3%.

Conclusion These results indicate that blood of patients with SLE contain antibodies to mitochondria. Among these antibodies, some may react to DNA as shown by the ability of soluble DNA to inhibit ELISA binding. While binding mitochondria, these antibodies differ from AMA found in primary biliary cholangitis since few reacted in the ELISA for M2 AMA. Since studies using flow cytometry have demonstrated that IgG positive EVs in the blood of patients contain mitochondria as shown by MitoTracker Deep Red, these results suggest that, like nuclei, mitochondria may be a subcellular organelle that can display autoantigenic determinants to form immune complexes in SLE.

AA-03 NEURONAL-SURFACE P-ANTIGEN (NSPA), TARGET OF ANTI-RIBOSOMAL P AUTOANTIBODIES, IS EXPRESSED IN PROXIMAL TUBULE EPITHELIAL CELLS (PTEC): POTENTIAL ROLE IN LUPUS NEPHRITIS

[1]Marcela Bravo-Zehnder, [1]Patricia Gajardo, [1]Daniela Valenzuela, [1]Tomás Toledo, [1]Ángel Jurado, [1]Fabián Segovia, [2]Gonzalo Méndez, [2]Carlos Vio, [1]Loreto Massardo*, [1,2]Alfonso González. *[1]Center for Cell Biology and Biomedicine (CEBICEM), Faculty of Medicine and Science, Universidad San Sebastián, Santiago, Chile; [2]Center for Aging and Regeneration (CARE), Faculty of Biological Science, Pontificia Universidad Católica de Chile, Santiago, Chile*

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Background Patients with systemic lupus erythematosus (SLE) and anti-ribosomal P (anti-P) antibodies may present lupus psychosis, cognitive impairment and lupus nephritis (LN). Our group provided a molecular mechanism to explain the neuronal alterations in SLE identifying the neuronal-surface P-antigen (NSPA) as an anti-P target involved in the regulation of synaptic glutamatergic transmission (Bravo-Zehnder *et al. A and R* 2015; Segovia-Miranda *et al. A and R* 2015). There is evidence suggesting that anti-P and anti-dsDNA antibodies have the potential to perturb the function of renal cells in addition to complement activation. The mechanism of anti-P association with lupus nephritis remains unclear. It is possible that direct anti-P interaction with cell surface components activates intracellular signalling pathways resulting in renal cell damage. As a first approximation to this hypothesis we study whether and where NSPA is expressed in the kidney.

Methods NSPA expression was assessed by RT-PCR, immunoblot and anti-NSPA immunohistochemistry in $C57^{WT}$ mice, ß-galactosidase (ß-gal) histochemistry staining in transgenic mice bearing a LacZ gene under the promotor of NSPA gene and anti-P staining in the surface of primary cultures of PTEC and the human kidney cell line HK2. NSPA polarized distribution was studied in Madin-Darby canine kidney (MDCK) cells transfected with NSPA-GFP expression plasmid. The pathogenic potential of anti-P antibodies was analyzed by immunizing $C57^{WT}$ and $NSPA^{KO}$ mice with recombinant P0 ribosomal bearing ($P0^{wt}$) or not the P epitope ($P0^{tr}$) and testing anti-P and anti-dsDNA presence, proteinuria and renal pathology by histochemistry and electron microscopy.

Results NSPA is expressed in PTEC cells and display a mainly apical distribution *in vivo* and *in vitro*, including the transfected MDCK cells. Both $P0^{wt}$ and $P0^{tr}$ generated high levels of anti-P antibodies and some mice also developed anti-dsDNA antibodies. Mice presented mild proteinuria at 6 months of immunization. $C57^{WT}$ and $NSPA^{KO}$ mice immunized with either $P0^{wt}$ or $P0^{tr}$ also displayed perivenular lymphocytic infiltration, a mild renal pathology change. In addition, $NSPA^{KO}$ mice, even in absence of anti-dsDNA, showed mesangial electron dense-deposits with discrete effacement of foot processes.

Conclusions NSPA is expressed in both PTEC and polarized MDCK epithelial cells with a polarized distribution that precludes direct interaction with circulating anti-P antibodies, which might access to this target only after glomerular filtration. Circulating anti-P can associate with mild kidney damage independently of NSPA expression, at least in non-lupus prone mice.

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AA-04 AUTOANTIBODIES TO M-PHASE PHOSPHOPROTEIN I (MPP-1: KIF20B) IN SYSTEMIC LUPUS ERYTHEMATOSUS

[1]May Y Choi*, [1]Eric Campbell, [1]Ann Clarke, [1]Michelle Jung, [1]Claire Barber, [2]Yvan St Pierre, [1]Marvin J Fritzler. *[1]University of Calgary, Cumming School of Medicine, Calgary, Alberta, Canada; [2]Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada*

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Background M-phase phosphoprotein (MPP-1), also termed kinesin interacting protein (KIF20B), is a 210 kDa protein that is highly expressed during cell division. Autoantibodies to MPP-1 were first described in approximately 25% of patients

with idiopathic ataxia, but recent studies have indicated that they are also found in systemic lupus erythematosus (SLE). The goals of this study were to determine the frequency of anti-MPP-1 in a local SLE cohort and then identify demographic, clinical, and serologic correlations.

Methods Patients fulfilling the American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria for SLE were enrolled in the SouThern Alberta Registry for Lupus EryThematosus (STARLET) cohort (Calgary, Canada). Demographic, clinical information (disease activity – SLEDAI-2K; damage – SLICC/ACR DI), and sera were collected at time of enrollment. Antibodies to MPP-1 were determined by an addressable laser bead immunoassay (ALBIA) utilizing an *in vitro* expressed MPP-1 cDNA construct inserted into a GFP vector (Clontech Laboratories Inc., Saint-Germain-en-Laye, France). ALBIA results were expressed as median florescence units (MFU) and a dilution of $\geq$1:500 MFU was considered highly positive. Univariable analysis was performed to determine associations between the prevalence of high positive anti-MPP-1 and demographic (age, sex, race/ethnicity), clinical features (SLEDAI-2K and SLICC/ACR DI total scores and subscales and neurological subscale of the ACR and SLICC Classification Criteria), medications, other autoantibodies (anti-dsDNA, extractable nuclear antigens, and anti-phospholipid antibodies).

Results One hundred and forty SLE patients were included; 89.3% were female with a mean age of 47.3 years (SD 16.3) and disease duration of 13.9 years (SD 11.6). The prevalence of high titre anti-MPP-1 was 15.0% (21/140) respectively. Univariable analysis demonstrated that high anti-MPP-1 positivity was associated with a higher total SLEDAI-2K score (Odds Ratio (OR), 1.1 [95% CI 1.0 to 1.3]), particularly with the serositis (OR 3.0, [95% CI 1.4 to 6.6]) and immunological subscales (OR 2.0, [95% CI 1.4 to 2.9]). High anti-MPP-1 positivity was also associated with anti-dsDNA (OR 5.5 [95% CI 1.8 to 16.6]), anti-SSA/Ro60 (OR 3.1 [95% CI 1.0 to 8.9]) and anti-phosphotidylserine/prothrombin complex (aPS/PT)-IgG (OR 3.6 [95% CI 1.1 to 11.5]).

Conclusions High titer anti-MPP-1 antibodies were common in this SLE cohort (15.0%) and may be associated with greater clinical and serologic SLE disease activity. A larger study is currently underway to more clearly delineate its role as a biomarker in SLE.

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AA-05 B CELL INTRINSIC IFNβ IS ASSOCIATED WITH AUTOANTIBODIES AND ACTIVE RENAL DISEASE IN SLE

[1]W Winn Chatham*, [1]Jennie A Hamilton, [1]Qi Wu, [1]PingAr Yang, [1]Bao Luo, [1]Shanrun Liu, [1]Jun Li, [1]Hui-Chen Hsu, [2]Inaki Sanz, [3]John D Mountz. *[1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Department of Medicine, Emory University; [3]Department of Medicine, Birmingham VA Medical Center, Birmingham*

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Background Dysregulated responses to type I interferons (IFNs) is a hallmark of autoreactive B cell development in SLE patients. High sera levels of type I IFN protein were recently shown to occur in the absence of increased circulating pDCs and in the absence of increased pDC IFNs, suggesting the likelihood of other important sources of type I IFN that may act on B cells. The present study determined the cellular source of IFNβ and its association with disease activities in SLE.

Methods Peripheral blood mononuclear cells (PBMCs) were obtained from 31 SLE patients meeting ACR 1997 revised criteria for SLE and 9 healthy controls. Intracellular IFNβ was determined using flow cytometry with FITC-anti-IFNβ mAb. Comprehensive clinical data was recorded for each SLE subject and the clinical data and laboratory analysis of B-cell intracellular IFNβ expression were collected in a double-blind fashion.

Results IFNβ was detected in various cell types including CD4 T cells, B cells and pDCs in PBMCs of SLE patients. Endogenous IFNβ in B cells was significantly higher than endogenous IFNβ in CD4 T cells and were equivalent to that seen in pDCs. Within B cells, there was a significant increase in endogenous IFNβ in all B cell subpopulations of SLE patients compared to healthy controls. The most significant increase was found in the $CD27^{+}IgD^{-}$ memory subpopulation. B-cell endogenous IFNβ was not a result of B-cell uptake of exogenous IFNβ as coculture of SLE B cells with HEK293 reporter cells resulted in induction of interferon stimulatory genes as determined by the secreted alkaline phosphatase assay. This was further blocked by an anti-IFNβ neutralization antibody. Interestingly B-cell endogenous IFNβ was highly correlated with clinical disease including renal disease and autoantibodies including anti-dsDNA, anti-Sm and anti-SSA. Strikingly, the highest correlation of IFNβ with clinical manifestations was observed in African-American patients. B-cell IFNβ expression was significantly correlated with $CD19^{lo}CD38^{hi}CD27^{+}$ plasma cell formation.

Conclusion Intracellular IFNβ production by B cells is a novel and important B-cell intrinsic factor that may be essential for B-cell development into autoantibody producing B cells. The present work suggests a need for future human lupus studies into type I IFN dysregulation that pioneer beyond the view of pDC produced IFNα. These results also provided a mechanistic basis for development of more effective therapies to target the high-affinity IFNβ or the enhanceosome components that promote its induction in a subgroup of lupus patients.

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Big Data Analyses

BD-01 E-GENES IDENTIFIED VIA TRANSANCESTRAL SNP MAPPING AND GENE EXPRESSION ANALYSIS REVEAL NOVEL TARGETED THERAPIES FOR AFRICAN-AMERICAN AND EUROPEAN-AMERICAN SLE PATIENTS

[1]Katherine A Owen, [1]Bryce N Aidukaitis, [1]Adam C Labonte, [1]Michelle D Catalina, [1]Prathyusha Bachali, [1]James Dittman, [1]Nicholas Geraci, [1]Sean Rouffa, [2]Hannah C Ainsworth, [2]Miranda C Marion, [2]Timothy D Howard, [2]Carl D Langefeld, [1]Peter E Lipsky, [1]Amrie C Grammer*. *[1]RILITE Research Institute, Charlottesville, VA, USA; [2]Wake Forest School of Medicine, Winston-Salem, NC, USA*

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Background Systemic lupus erythematosus (SLE) in African-Americans (AA) is more prevalent, more severe and associated