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CORRELATION BETWEEN INTERSTITIAL CD8+ T CELL INFILTRATION AND FIBROTIC PROCESSES IN A MOUSE MODEL OF LUPUS NEPHRITIS

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Background Tubulo-interstitial damage during lupus nephritis (LN) is associated with poor renal prognosis in the long run. Here, we describe the progression of tubulo-interstitial fibrosis and immune cells infiltration with emphasis on CD8+ T cells, in parallel with renal outcomes in a mouse lupus model.

Methods We collected blood, urine and kidneys from 39 B6/Sle1.Sle2.Sle3 lupus-prone mice, before disease onset and at different stages of disease progression. RNA was extracted from kidneys and hybridized on Mouse Gene 2.0 ST exon arrays. Histopathological scores (NIH Activity and Chronicity Indexes) and digital quantification of fibrosis, IgGk deposits, CD8+ and CD3+ T cells were performed on total kidney. Renal CD8+ T cell phenotypes were determined by flow cytometry. Plasma urea and albuminuria were measured by immunoenzymatic assays.

Results IgGk deposits, CD8+ T cell infiltration and interstitial fibrosis increase with the progression of renal disease, evaluated by histopathological scores and plasma urea. Further, digital quantifications allowed us to identify a significant correlation ($r=0.45$, $p=0.011$) between local CD8+ T cell population and fibrosis, while total CD3+ cells population and IgGk deposits did not display such association. Moreover, characterization of CD8+ T cell subpopulations showed that fibrosis is more specifically linked to effector functions of CD8+ T cells. Transcriptomic analyses supported this association, with a high correlation coefficient between mean expression of effector functions transcripts and the presence of a fibrotic signature ($r=0.92$, $p<0.0001$).

Conclusions Our results support the association between CD8+ T cell tubulo-interstitial infiltration and renal outcomes in a mouse lupus model. Further, a strong correlation is identified between effector functions of CD8+ T cells and fibrotic processes, opening new avenues of research in the pathogenesis of LN.

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P105

EXPRESSION DIVERSITY OF INTERFERON-STIMULATED GENES IN PERIPHERAL BLOOD CELLS FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background A central facet of the immunopathogenesis of systemic lupus erythematosus (SLE) is the activation of interferon (IFN) signalling. In SLE patients, several types of IFNs are upregulated as well as the genes that are stimulated by IFNs. Sustained expression of interferon-stimulated genes (ISGs) may have adverse effects including altered T cell function, tissue inflammation and organ damage. The distinct association of the IFN gene signature to SLE disease activity is still uncertain.

Methods Peripheral blood samples from 34 SLE patients and 15 healthy controls were collected in PAXgene tubes. RNA from peripheral blood cells (PBCs) was extracted using the PAXgene Blood RNA Kit. The mRNA transcripts of 105 ISGs were measured using the multiplexed Nanostring nCounter Gene Expression platform. Bioinformatics and statistical analysis were performed using nSolver and SPSS.

Results We found that SLE patients had significantly higher expression levels of a wide range of ISGs, as compared to healthy controls. The 5 most upregulated ISGs in PBCs were IFI27, IFI44L, USP18, RSAD2 and ISG15. Using principal component analysis we identified two subsets of ISGs of which one included the mentioned top-five expressed ISGs; this gene cluster consisted of ISGs with predominantly antiviral functions and STAT1/STAT2, and did not associate with clinical disease activity or history of nephritis in SLE patients. Instead these genes associated with anti-SSA/SSB antibodies and PBC expression of PDCD1 and LAG3, which are markers of CD8 T cell exhaustion. The second group of ISGs associated with clinical disease activity, STAT3 and NFkB expression.

Conclusions Our results indicate the presence of ISG subsets that are differentially associated to clinical, serological and immunological features of SLE. Thus, the IFN gene signature in SLE may consist of a highly complex network of ISGs that can be clustered, and each in their way contribute to the pathogenesis of SLE. Also, this challenges the construction IFN gene signatures and scores with respect to what purpose they are intended for.

P106

PENTAMERIC, BUT NOT MONOMERIC C-REACTIVE PROTEIN, LIMITS THE SNRNP-IMMUNE COMPLEX TRIGGERED TYPE I INTERFERON RESPONSE: IMPLICATIONS FOR LUPUS PATHOGENESIS

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Background Systemic Lupus Erythematosus (SLE) is an autoimmune systemic disease affecting multiple organs and which is characterized by autoantibodies directed against nuclear constituents. Common autoantibody targets include double-stranded (ds) DNA and small nuclear ribonucleoproteins (snRNPs; i.e. U1-snRNP). Uptake of immune complexes (ICs) by plasmacytoid dendritic cells (pDCs) can activate endosomal toll-like receptors (TLRs) such as TLR-7 and TLR-9 if nucleic acids are present in the ICs. Such activation is dependent on IC internalization by Fcγ receptor type IIa (FcγRIIa), and results in production of type I interferons (IFNs), a hallmark of SLE and a target for therapeutic interventions. The acute phase protein C-reactive protein (CRP)

binds FcγRs, as well as some nuclear proteins, including snRNPs. Pentameric (native) CRP has previously been suggested to inhibit production of IFNs in peripheral mononuclear cells (PBMCs) in response to ICs formed by autoantibodies against snRNP, an effect which was further investigated herein.

Methods PBMCs or magnetically (MACS) purified pDCs were retrieved from whole blood of healthy volunteers. Type I IFN gene transcription and production was stimulated by addition of snRNP containing ICs ± pentameric CRP (pCRP) or monomeric CRP (mCRP) in different sequential order. IC formation was achieved through simultaneous addition of snRNP and bulk IgG, retrieved from an SLE patient with high levels of snRNP autoantibodies, directly to the cells. Type I IFNs and inflammatory cytokines were investigated using quantitative PCR, ELISA and cytometric bead array, and cells responsible for production of the IFNs were characterized using flow cytometry. For statistics a two-tailed t-test was performed.

Results pCRP had an inhibitory effect on the IFN gene expression in PBMCs after incubation with ICs, $p=0.044$ for IFNα4 and $p=0.047$ for IFNβ at the 4h time-point compared to IC only. pCRP also showed a dose-dependent inhibitory effect on the type I IFN production in the cells. The monomeric form of CRP showed modest or no effect on IFN levels, $p=0.82$ for IFNα4 and $p=0.58$ for IFNβ at the 4h time-point compared to IC only. A pre-incubation of the cells with pCRP increased the inhibitory effects compared to simultaneous addition of pCRP and ICs, suggesting that initial binding to the cells is a critical step for inhibition. Flow cytometry suggested that pDCs are the main producer of the type I IFNs. In addition, pCRP seems to have a more general inhibitory effect on type I IFNs, as seen in the reduction of IFN production in response to the TLR-9 ligand CpG.

Conclusions pCRP has a distinct inhibitory effect on type I IFNs, which is largely not seen for the dissociated form of CRP (mCRP). The more general inhibitory effects shown by pCRP highlights its immune regulatory function in pathologies characterized by high production of type I IFNs. The identity of the initial receptors responsible for pCRP mediated effects, as well as of the involved signaling pathways, will be further investigated.

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INTERFERON-INDUCED METABOLIC PERTURBATIONS SHAPE THE INFLAMMATORY STATUS OF HUMAN MONOCYTES: IMPLICATIONS FOR INNOVATIVE THERAPEUTIC ENGINEERING IN SLE AUTOIMMUNITY

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Immune cells have unique metabolic requirements to support the energetic and biosynthetic burden during their activation. Delineation of the metabolic tuning of immune cells could lead to novel strategies in treating metabolically-

demanding processes including autoimmune diseases. Among innate effectors, monocytes have a distinct role in systemic lupus erythematosus (SLE) pathogenesis. We have previously described robust type-I interferon (IFNα) signaling in patients with SLE. IFNα-stimulated monocytes from healthy individuals (IFN-Mo) develop mitochondrial hyperpolarization and increased oxidative stress resembling SLE monocytes (SLE-Mo).

Here we sought to delineate the metabolic repercussion of IFNα-mediated signaling that could explain metabolic shifts pertaining to autoimmunity. To this end, we combined transcriptomic data with metabolic flux analysis (Seahorse technology) and Gas Chromatography (GC-MS) in healthy monocytes, IFN-Mo and SLE-Mo. Our preliminary results indicate an increased, glucose-dose dependent glycolytic flux in IFNα-treated healthy monocytes recapitulating the SLE-Mo phenotype. Blockade of hexokinase 2 (HK-2)-dependent glycolysis with the use of 2-DG inhibitor attenuated proinflammatory cytokine secretion and the expression of surface markers characteristic of activated monocytes, supporting the deregulated metabolic profile in SLE autoimmunity.

Combination of these data with targeted metabolomics (LC-MS) analyses and the application of pathway-specific inhibitors are implemented *in vitro* to reverse the inflammatory state of SLE monocytes. Together, our data are expected to yield unique insights into the role of immunometabolism in SLE and the potential use of metabolites as novel therapeutic targets in autoimmunity.

P108

OXIDATIVE STRESS IN NK CELL AND ITS CORRELATION WITH EXPRESSION OF KILLER IMMUNOGLOBULIN RECEPTORS IN SLE PATIENTS

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Background Oxidative stress i.e. accumulation of reactive oxidative species has been found to be implicated pathogenesis of many autoimmune diseases including systemic lupus erythematosus. Although our body has natural process of scavenging reactive oxidative species but whenever balance inclines towards accumulation, oxidative stress begins to build in. These accumulated ions lead to damage at cellular level and at molecular levels also. In our NK cell specific study we evaluated oxidative stress and expression level of killer immunoglobulin receptors. Killer cell immunoglobulin like receptors binds to mhc class 1 receptors. They work in antagonistic manner they are either activating for NK cell activity or inhibiting. So the balance between two categories is critical for self tolerance. We have evaluated expression level of kir2dl4 which binds to HLA-G ligand and activating in nature and KIR3DL1 on other hand interacts with HLA-Bw4 and prevent NK cell killing of healthy cells.

Methods *Lupus Patient and healthy subjects* Lupus patients are enrolled from outpatient department (OPD) of rheumatology clinic, PGIMER, Chandigarh.

Flowcytometric analysis PBMC isolated were incubated with antibodies conjugated to APC, PE, PerCP/cy5.5 for surface staining of antigens cd56, kir2dl4 and kir3dl1. DCFDA dye based analysis was al done for estimation cellular ROS levels.