PLASMA Blast proliferation is associated with toll like receptor 7 polymorphisms and upregulation of type I interferon, contributing to the antibody production in antiphospholipid syndrome

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Background: Antiphospholipid antibodies (aPL) as pathogenic autoantibodies in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) are supported by a number of clinical, ex vivo and animal studies. Nevertheless, aPL are not eliminated by corticosteroid administration or immunosuppression. Novel therapy targeting aPL production is currently unmet needs, in contrast, little is known on its pathological mechanism.

Objectives: This study aimed to clarify the mechanism of aPL production by lymphocyte subset analysis, genomic analysis and ex vivo experiments.

Methods: B cell and T cell subsets, a total of 21 subsets, were evaluated in peripheral blood mononuclear cells (PBMC) of 26 primary APS (PAPS), 18 SLE-APS and 11 healthy controls by flow cytometry. Genomics, Lausanne, Switzerland

Results: In PAPS and SLE-APS patients, plasmablasts, TH2 cells and TH17 cells were increased while pre- and post-switched memory B cells, regulatory B cells and regulatory T cells were decreased compared to healthy controls. Genomic analysis revealed that the increase of plasmablasts (p=0.032) and the decrease of memory B cells (p=0.013) were more pronounced in patients with a risk allele of SNP in toll like receptor 7 (TLR7) gene (rs3853839). IFN score was significantly higher in TLR7 SNP risk allele carriers, confirming the downstream signaling of TLR7 (p=0.029). Ex vivo experiments showed that aPL, including anti-cardiolipin (aCL), a-glycoprotein I (aGPI) and HLA class II complexes, -IgG and -IgM were present in the culture supernatant of CD19+CD20− depleted PBMC from APS patients, but not in that of CD19+CD20− depleted cells.

Conclusions: Our data indicate an important role of plasmablasts in the production of aPL. Furthermore, plasmablast proliferation was associated with TLR 7 and type I IFN, suggesting a common pathophysiology in SLE and APS. Targeting plasmablasts might be a novel, immunological therapeutic approach in the treatment of APS.

REFERENCE:
**Objectives:** We hypothesised that hyperuricemia affects neutrophil chemotaxis in CKD patients. Furthermore, we made use of a novel mouse model of chronic uric acid nephropathy.

**Methods:** **Human study:** Serum was collected and neutrophils isolated from CKD patients or healthy subjects. Serum BUN (blood urea nitrogen), creatinine and uric acid levels were measured. Neutrophil transwell assays were carried out and the number of migrated neutrophils towards FMLP, human IL-8 determined by flow cytometry. **Animal study:** Six week old Abc-creERT2;Glut9 flox/flox mice (ki/ki) and mice with active Cre (+/+) were injected with tamoxifen. The ki/ki mice received either a high fat diet with Insosine (HFD+ino) to induce hyperuricemia-associated CKD or a Chow diet with Insosine (Chow+ino) to induce only hyperuricemia without CKD. Control +/+ mice either received HFD+ino or Chow+ino diet. After two weeks, all groups were injected either with MSU crystals or vehicle into a preexisting air pouch, a mouse model for acute gouty arthritis. After 12 hours, neutrophil infiltration and the extent of inflammation were assessed via flow cytometry, ELISA, and cobrometric assays.

**Results:** **Human study:** Compared to healthy subjects, CKD stage 5 patients presented with significant higher levels of serum BUN (14.1 vs 52.1 mg/dl, p<0.001), creatinine (1.5 vs 9.3 mg/dl, p=0.001) and UA (2.3 vs 10.3 mg/dl, p<0.001). Neutrophils from CKD patients showed an impaired migratory ability due to the down-regulation of the adhesion molecules P-Selectin and uLigintegriin.

**Animal study:** Two weeks post-HFD+ino, ki/ki mice developed hyperuricemia-associated CKD (serum UA: 10–14 mg/dl; BUN: 80 mg/dl), whereas the ki/ki mice on Chow+ino diet became hyperuricemic without CKD. Control +/+ mice on both diets did not develop hyperuricemia nor CKD. Interestingly, the number of infiltrating neutrophils into the air pouch was reduced in hyperuricemic ki/ki mice with CKD as well as in hyperuricemic ki/ki mice without CKD compared to +/+control mice. We observed less inflammation indicated by decreased IL-1γ, TNF, CXCL1 and myeloperoxidase levels, and a down-regulation of adhesion molecules on infiltrated neutrophils in hyperuricemic ki/ki mice with CKD compared to +/+control mice.

**Conclusions:** Our data show that neutrophils from CKD patients are less able to migrate, which was consistent with data from our novel mouse model demonstrating that hyperuricemia impairs neutrophil chemotaxis in MSU crystal-induced inflammation. This indicated that the mechanism for defective neutrophil migration might be responsible for the lower incidence of acute gouty arthritis in hyperuricemic CKD patients.

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**SADRA TREATMENT RESPONSE STUDIES IN 800 HUMAN BLOOD SAMPLES REVEALS SHARED AND UNIQUE EXPRESSION PROFILES ACROSS SEVEN SYSTEMIC AUTOIMMUNE DISEASES**

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**Background:** Systemic autoimmune diseases (SADs) are chronic inflammatory conditions with limited treatment options. Although SADs encompass different clinical diagnoses, many of them share common pathophysiological mechanisms and have similar clinical manifestations. Therefore, defining a precise diagnosis and consequently an appropriate treatment is complex.

**Objectives:** We aimed to identify characteristic expression profiles for patients diagnosed with different SADs and find specific biomarkers for each disease based on whole blood RNA-seq data.

**Methods:** As part of the ongoing IMI PreciseSADS project we generated and analysed globin-depleted, polyA-selected RNA-seq data from an initial subset of 800 peripheral blood samples of healthy controls and patients with seven different SADs: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), Sjögren’s syndrome (SSj), mixed connective tissue disease (MCTD), undifferentiated connective tissue disease (UCTD) and primary anti-phospholipid syndrome (PAPS). Differential gene expression analysis was performed with DESeq2 and biomarker discovery was achieved using linear support vector classifier-based feature elimination and logistic regression-based estimator with cross-validation.

**Results:** We identified unique and common genes that were differentially expressed between controls and each of the seven SADs. The greatest extent of transcriptional dysregulation was found in SLE and MCTD patients, while UCTD, SSc and RA showed lowest differentially expressed genes. We found large and statistically significant overlaps between the lists of differentially expressed genes for each disease, with SLE, MCTD, SSj and UCTD showing most pronounced similarity at the gene expression level. The overlapping genes were enriched in interferon signalling pathway and the classical complement pathway. Low overlap was found between SSj and RA, and SSj and SSc.

We also looked for unique gene expression patterns in each disease with the aim of identifying potential biomarkers. We were able to define gene signatures differentiating between SADs and controls and also between SAD pairs.

**Conclusions:** Even though we were able to identify a limited number of disease specific signature genes, there are extensive and statistically significant overlaps in gene expression profiles of the seven investigated SADs. Similar to the clinical manifestations, the data presented here suggest that also on the molecular level, these diseases share a large portion of their pathophysiology. Work is ongoing to expand the dataset for confirmation of these preliminary data.

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**RNA-SEQUENCING OF 800 HUMAN BLOOD SAMPLES REVEALS SHARED AND UNIQUE EXPRESSION PROFILES ACROSS SEVEN SYSTEMIC AUTOIMMUNE DISEASES**