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 Alb-creERT2;Glut9^{lox/lox} mice (ki/ki) and mice without active Cre (+/+) were injected with tamoxifen. The ki/ki mice received either a high fat diet with Inosine (HFD+Ino) to induce hyperuricemia-associated CKD or a chow diet with Inosine (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 colorimetric 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 $\alpha\beta$ Integrin.

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 neither 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-1 β , TNF α , 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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Big data in pre-clinical research

OP0359 EXPLORATION OF T-CELL SIGNATURES FOLLOWING TCR STIMULATION USING SINGLE CELL RNA-SEQ TO INFORM TREATMENT RESPONSE STUDIES IN RHEUMATOID ARTHRITIS

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Background: For rheumatoid arthritis (RA), as with many other rheumatic diseases, the importance of determining which therapy will work best, early in disease, to prevent further progression, is an important area of research. Progress in treatment response has been limited, possibly due to the complex interplay between various cell types. As such, specific T-cell signatures, determined by single cell RNA-Seq (scRNA-Seq), could be predictive of future response to treatments such as anti-TNF biologic therapies.

Objectives: Our aim was therefore to determine the optimal study design and to assess the potential of scRNA-Seq to identify T-cell signatures under resting and stimulated conditions to inform future studies.

Methods: Primary CD4 +T cells were either stimulated using anti-CD3/CD28 beads or subjected to the same conditions without stimulation for 4 hours. Single cells were isolated using the 10X Genomics Chromium Controller with a target recovery of 6000 cells. Each scRNA-Seq library was sequenced on 4 Illumina HiSeq 4000 lanes (~200K reads/cell) and processed using the cellranger pipeline. Further quality control and cluster analysis was performed using Seurat.

Results: For the unstimulated sample 5,586 cells were recovered and after quality control and filtering, 5,387 cells remained. Similarly, for the stimulated sample, 4,621 cells were recovered and 4,473 remained. This resulted in an average of 1094 and 1456 genes per cell. Similar clusters were seen after downsampling the stimulated dataset to 1 lane (~379M reads, ~82K reads/cell), suggesting that

CD4 +T cells are defined by large gene expression changes rather than subtle variations, consistent with protein expression data. Cluster exploration allowed the identification of several typical CD4 +T cell populations, including naïve, helper and regulatory. Furthermore, alignment of the two conditions in Seurat, identified classical and non-classical markers of activation, such as CD69, CCR7, MYC and PIM3. Finally, the relative cluster location and the expression of indicative markers suggested evidence of a progression from a naïve cell state to an 'active' effector state.

Conclusions: This data has provided important insights into future study design and confirmed the potential of scRNA-Seq to identify T-cell signatures. Importantly, despite obvious expression changes, cluster identity was maintained between stimulatory conditions. This implies it is possible to directly compare scRNA-Seq expression profiles between patient samples showing different disease activity without confounding the conclusions and enable the use of scRNA-Seq to investigate its predictive potential in RA treatment response. We are therefore in the process of expanding this work to study patient samples and different cell types. For example we have already generated similar data for monocytes on 3 RA samples and 3 healthy samples.

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OP0360 RNA-SEQUENCING OF 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 antiphospholipid 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 fewest differentially expressed genes. We found large and statistically significant overlaps between the lists of differentially expressed genes for each disease, with SLE, MCTD, SJS 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 SJS and RA, and SJS 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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