were sacrificed at either 7, 14 or 30 days after the last injection and blood, aorta and heart specimens were harvested. A pathologist (OT), blinded to treatment groups, examined aortic root and adjacent myocardium specimens and assigned a score for the degree of inflammation (0-6).

Results: Seven MCD-CAWS mice died unexpectedly within 24 hours of the first CAWS injection. Autopsies reviewed marked liver sinusoidal dilation in all and patchy necrosis of the liver in 4. Similar, liver findings were seen in 75% of MCD-CAWS after 7 days, but not in other groups. MCD-CAWS mice had higher systemic IL-6 compared to WT-CAWS at both 7 days (208 ± 21 vs 152 ± 12 pg/ml; $p < 0.001$) and 14 days (226 ± 49 vs 169 ± 19 pg/ml; $p = 0.04$), but not at 30 days. By 14 days mice in MCD-CAWS had significantly higher serum INF γ compared to WT-CAWS mice (97 ± 4 vs 87 ± 7 pg/ml, $p = 0.0086$) which remained persistently elevated even at 30 days (103 ± 8 vs 91 ± 4 pg/ml; $p = 0.002$). TNF α was higher in MCD-CAWS compared to WT-CAWS at 7 days (258 ± 8 vs 243 ± 6.5 pg/ml, $p = 0.0016$), 14 days (266 ± 14 vs 239 ± 6 pg/ml, $p < 0.001$) and 30 days (326 ± 52 vs 256 ± 8 pg/ml, $p = 0.002$). The average aortic root inflammatory score was non-significantly higher in the MCD-CAWS group compared to WT-CAWS at both 7 days (1.5 vs 1.3) and 14 days (3.7 vs 2.4).

Conclusion: Mast cell deficiency resulted in higher systemic levels of IL-6, TNF α and INF γ in the CAWS mouse model of Kawasaki's disease. Similarly, mast cell deficiency resulted in more intense inflammation at the root of the aorta in this model. These results support the novel concept that mast cells play a protective role in reducing the initial systemic inflammatory response in Kawasaki's disease.

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THU0507

TYPE I INTERFERON SCORE AND INTERFERON INDUCED MEDIATORS CXCL10 AND NEOPTERIN ARE CORRELATED WITH DISEASE ACTIVITY IN JUVENILE DERMATOMYOSITIS

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Background: Interferons (IFNs) seem to play an important role in the pathogenesis of juvenile dermatomyositis (JDM). Our group previously reported that expression of both type I and type II IFN related genes is increased in muscle biopsies of JDM patients and correlates with histological and clinical features of the disease.

Objectives: The aim of this study was to investigate expression of interferon regulated genes (IRGs), as well as serum levels of two type I and type II IFN induced chemokines (CXCL9, CXCL10) and neopterin in peripheral blood of JDM patients and to assess their correlations with clinical and laboratory findings.

Methods: We collected 189 blood samples from 39 JDM patients at different time points during follow-up. In 11 patients we obtained the first blood sample at time of muscle biopsy. We measured expression of type I IRGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1), IFN γ and type II IRGs (CXCL9, CITA, IDO1) by quantitative PCR (qPCR) and calculated a type I and type II IFN score for muscle and blood samples; serum

levels of CXCL9, CXCL10 and neopterin were analyzed by ELISA. Ten healthy subjects were used as controls (HC). At each visit, the following clinical data were recorded: physician's global assessment (PGA) of disease activity VAS (Visual Analogue Scale), cutaneous VAS, Cutaneous Assessment Tool (CAT) activity score, Childhood Myositis Assessment Score (CMAS), serum levels of creatine phosphokinase (CK, IU/l), presence of myositis specific or myositis associated antibodies (MSA/MAA), prednisone (or equivalent) dose (mg/kg/daily), ongoing immunosuppressive medications.

Results: Serum levels of CXCL9 were significantly correlated with muscle expression of IFN γ and type II IFN score. The correlation of CXCL10 levels with muscle type I and type II IFN score was weaker. Muscle expression of CXCL9 and CXCL10 correlated with serum levels of these chemokines. Type I IFN score in blood of JDM patients was increased compared to HC and significantly correlated with PGA, cutaneous VAS, CAT activity score. Serum levels of CXCL9 and CXCL10 were significantly higher in JDM patients compared to HC. MSA positive JDM patients showed higher levels of CXCL9 and CXCL10 compared to MSA negative patients. CXCL10 levels correlated with PGA and CMAS, but not with cutaneous disease activity. CXCL9 showed no significant association with the evaluated clinical features. Neopterin levels significantly correlated with PGA, cutaneous VAS, CAT activity score and CMAS.

Conclusion: Our findings indicate that expression of IRGs, measured as type I IFN score, and serum levels of CXCL10 and neopterin reflect specific features of disease activity in JDM, supporting their role as valuable disease biomarkers.

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THU0508

CHANGES IN MIR-17-92 CLUSTER EXPRESSION LINK SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS, MONOCYTE-TO-MACROPHAGE DIFFERENTIATION, AND INTERFERON REGULATION

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Background: MicroRNAs (miRNAs) are small noncoding RNAs which post-transcriptionally regulate gene expression. The miR-17-92 cluster is well studied in cancer biology and cellular differentiation; its overexpression has been found to serve a major oncogenic role in the targeting and downregulation of tumor-suppressive pathways, such as PTEN or TGF β . Our previous work identified several members of the cluster – miR-18, miR-19a/b, miR-20a, and miR-92a – with significantly higher levels in monocytes from patients with active Systemic Juvenile Idiopathic Arthritis (SJIA). SJIA is a chronic inflammatory disease of childhood with features of autoinflammation, and innate immune cells including monocytes have important roles in disease pathogenesis. Children with SJIA are at risk for life-threatening complications including Macrophage Activation Syndrome (MAS), an episode of overwhelming inflammation characterized by macrophage proliferation and driven by IFN γ .

Objectives: Characterize the regulation of the miR-17-92 cluster, define key targets, and determine the cluster's role in inflammation and SJIA.

Methods: MiRNA levels were examined in THP-1 cells, as well as primary human monocytes isolated from healthy donors over the course of the monocyte to monocyte-derived macrophage (MDM) transition. MiR-17, miR-19a, and miR-20a were overexpressed via transfection in CD14+ monocytes for 2 days. Transcriptional profiles were performed using Ampliseq Transcriptome and the Ion Torrent S5 system and analyzed using AltAnalyze. Potential targets of the miR-17-92 cluster determined from sequencing analysis were then validated via dual-luciferase reporter assay.

Results: Neither blood monocytes nor fully differentiated THP-1 cells showed significant changes in miR-17-92 levels under standard polarization conditions, including M1, M2a, and M2b conditions, or IL-6 and IL-10 stimulation. The most sizable changes in miR-17-92 levels were found during monocyte to macrophage transition. Interestingly, primary monocytes showed increases in miR-17-92 levels within the first 48 hours of differentiation towards MDM, variable by miRNA and experiment, similar to that seen in SJIA monocytes. In contrast, both PMA-differentiated THP1 cells and fully differentiated MDMs showed decreased miR-17-92 compared to undifferentiated monocytic cells. MiR-17-92 was overexpressed in vitro in primary monocytes to model these early transition changes. Genome-wide transcriptional profiling showed an upregulation of genes involved in Type I and II Interferon pathways, including response to interferon-alpha (adjusted $p=2.71 \times 10^{-12}$) and interferon-gamma (adjusted $p=7.81 \times 10^{-9}$). Analysis of genes significantly downregulated by miR-17, miR-19a, or miR-20a identified several putative and previously validated miR-17-92 cluster targets, including ATG5, IFRD2, JAK1, PPARG, and PTPN2 which have interferon-regulatory functions. Dual-luciferase reporter assay experiments support that these genes are direct targets of miR-17, miR-19a, and/or miR-20a.

Conclusion: MiR-17-92 cluster members demonstrate initial increase followed by subsequent decrease in expression during 2-week human monocyte to macrophage differentiation. Overexpression of miR-17-92 miRNAs upregulates Type I and II interferon pathway genes, and these miRNAs target multiple genes involved in regulating interferon signaling and/or inflammatory response. Taken together, miR-17-92 cluster overexpression in SJIA monocytes may suggest a more differentiated phenotype, and contribute to IFN γ sensitivity and risk for MAS.

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THU0509

MONOCYTES PROTEOMIC PROFILE OF PATIENTS WITH DIFFERENT AUTOINFLAMMATORY DISEASES: A NEW APPROACH TO CHARACTERIZE THESE DISEASES

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Background: Autoinflammatory diseases are a group of inherited diseases characterized by early onset and systemic inflammation. These pathologies are caused by mutations in genes involved in the regulation of innate immune response with a consequent inflammatory phenotype. The most common genetically defined periodic fevers are Familial Mediterranean Fever (FMF), Cryopyrin-associated periodic syndromes (CAPS), TNF receptor-associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD/HIDS).

Some patients show clinical features similar to autoinflammatory diseases but no genetic mutation has been found.

Objectives: Our aim is to evaluate the differences in the expression of proteins or pathway in monocytes, and plasma metabolites in patients with autoinflammatory diseases compared with healthy subjects to clusterize and better understand the mechanisms underlying different genetically defined disorders and try to characterize the genetically undefined pathologies.

Methods: Monocytes, purified from peripheral blood and incubated with or without LPS, were collected from patients and healthy donors; samples have been processed by iST protocol. Each digested sample was analyzed by high-resolution liquid chromatography and tandem mass spectrometry (LC-MS/MS) based on Orbitrap technology. The quantification strategy is a label-free approach (LFQ) available in MaxQuant suite.

Results: Here we identified a median of about 5000 proteins from the monocyte samples of each 4000 are quantified by LFQ approach. PCA analysis and Person's correlation show good reproducibility of data and a good separation between the different groups. The data were then

submitted to an appropriate statistic. T-Tests highlighted differentially expressed proteins and through Cytoscape with the ClueGo app we obtained the differently regulated pathways in the different conditions. It has also been constructed, starting from significative proteins, a network, related to disease using the information of String Disease db. We observed that the expression of proteins is differently enriched according to the different conditions. For each autoinflammatory disease, a list of significantly modulated proteins was obtained: some of which are already known to be related to the disorders, while others have not yet been described. In FMF, MEFV, RhoA and some related proteins were significantly up-regulated together with genes linked to the interferon pathway. In TRAPS relevant proteins turn up related to the maintenance of Golgi and cellular trafficking. The bioinformatics analysis allows us to better understand the functional interaction between these monocytes proteins and map which are involved in the diseases.

Conclusion: Here, we addressed how a high-resolution proteomics approach could be used to better understand the biology of autoinflammatory diseases. The characterization of a broad spectrum of proteins and their interaction network will allow us to identify new biomarkers for the different pathologies and better comprehend and recognize the genetically undefined disorders.

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THU0510

NEUTROPHIL FUNCTION IN PEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: While the role of adaptive immune system in pathogenesis of systemic lupus erythematosus (SLE) has been well characterized, there is paucity of literature on innate immunity in these patients. There are limited data on neutrophil function in pediatric lupus. In this study we analyzed neutrophilic functions in a cohort of pediatric lupus from North India.

Objectives: To evaluate phagocytic and oxidative burst activity of neutrophils in patients with pediatric-onset SLE

Methods: This prospective study was carried out at a tertiary care center in North India in patients with SLE during the period July 2017 to December 2018. Diagnosis of lupus was based on SLICC 2012 criteria and patients who had disease onset below 18 years were included. Controls were age matched children attending the outpatient clinic of department. Disease activity was measured by SELENA-SLEDAI score. Phagocytic activity was estimating using the pH Rhodo *Escherichia coli* (E coli) BioParticles kit by flow cytometry. Phagocytic function was measured as% phagocytic activity of neutrophils, delta mean fluorescent intensity (Δ MFI), and stimulation index (SI). Oxidative burst activity was performed by Dihydrorhodamine (DHR) flow cytometry assay and% positivity neutrophils, Δ MFI, and SI were calculated.

Results: Eighty-seven children with lupus (63 girls; 24 boys) comprised the study group. 44 healthy controls were also enrolled. Disease onset was 8.52 ± 2.95 years whereas age at enrolment was 12.34 ± 5.67 years. Phagocytic activity in neutrophils SLE and controls were $76.59 \pm 20.70\%$; $91.30 \pm 4.47\%$; $p < 0.001$ respectively. Δ MFI phagocytosis in patients with SLE and control were 0.09 (0.05-0.16), 0.18 (0.15, 0.22); ($p = 0.002$) respectively. SI of phagocytosis in patients with SLE and controls were 2.79 (1.92, 3.93); 5.00 (4.50, 6.12); $p < 0.001$. SLEDAI score was negatively correlated with phagocytic function in neutrophils in patients with SLE. Oxidative burst activity of neutrophils in patients with SLE and controls in form of%positivity on neutrophils were $84.03 \pm 17.36\%$, $92.26 \pm$

5.49; $p < 0.001$ respectively. Δ MFI and SI on DHR between patients and healthy controls were comparable. There was no difference in oxidative burst activity between active and inactive SLE patients. Two patients showed a double peak in DHR flow cytometry consistent with mosaic pattern associated with a probable carrier state for X-linked chronic granulomatous disease. Both oxidative burst activity and phagocytic activity did not show significant correlation with the dose of prednisolone.

Conclusion: This study suggests that there is impaired neutrophil function in childhood SLE and also there may be a correlation between neutrophil dysfunction and disease activity. The *E. coli* based phagocytic functions of neutrophils were significantly reduced in pediatric SLE patients compared to healthy controls. Phagocytic activity of neutrophil was significantly lower in patients with disease activity and coexistent infection in patients with pediatric SLE. Oxidative burst activity was reduced in patients with pediatric SLE compared to healthy controls. However, there was no significant correlation of oxidative burst activity to the age, disease activity and coexistent infections.

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Abstract THU0510 – Figure 1. Phagocytic functions in patients with active SLE versus inactive disease

Abstract THU0510 – Figure 2. Correlation of SLEDAI and phagocytic activity

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THU0511

COMPARISON OF PAXGENE AND TEMPUS WHOLE BLOOD RNA COLLECTION AND ISOLATION SYSTEMS FOR THE QUANTIFICATION OF TYPE I INTERFERON-STIMULATED GENE EXPRESSION

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Background: Type I interferons (IFN) have important roles in many pediatric and adult rheumatic diseases and are a new therapeutic target for which several "anti-interferon (anti-IFN)" treatments are currently in use or in development. Since the direct detection of these proteins in biological samples has proved challenging, indirect methods are often used to infer the presence of type I IFN. Most commonly this involves quantification of the relative expression of interferon-stimulated genes (ISGs) that are used to calculate an interferon score (IS) (1). This score has been used for example to assess type I IFN activity in pediatric patients with type I interferonopathies, systemic lupus erythematosus, dermatomyositis and systemic juvenile idiopathic arthritis (2). Both qPCR and Nanostring technology have similar sensitivity and reproducibility for IS determination (3). The use of different whole blood RNA collection systems on the IS have not been evaluated however despite evidence of method-dependent changes in gene expression (4).

Objectives: The aim of the study was to compare expression of six common ISGs (*IFI27*, *IFI44L*, *IFIT1*, *ISG15*, *RSAD2*, *SIGLEC1*) and the corresponding IS in RNA derived from two commonly used whole blood RNA collection systems (PAXgene and Tempus).

Methods: Whole blood was collected from ten healthy individuals (median age 25.5 years) in sodium heparin tubes and incubated without or with recombinant human interferon alpha 2b (rhIFN α , 2 IU/ml, 4 hrs, 37C°, 5% CO₂). Next, samples were divided between PAXgene (PreAnalytiX, Becton Dickinson) and Tempus (Applied Biosystems) tubes and RNA was isolated according to the manufacturer's protocols. cDNA was synthesized (500ng input RNA; qScript cDNA synthesis kit) and ISG expression measured on a QuantStudio 6 Real-Time PCR instrument using a TaqMan Fast Advanced Assay. For each ISG, expression was normalized against the geometric mean of two housekeeping genes (18s rRNA and HPRT1) and calculated using the formula $2^{-\Delta\Delta Ct}$. Relative gene expression is reported as the normalized expression of each ISG divided by the median of normalized expression of the same ISG in unstimulated samples. The median relative expression of all six ISGs was used to calculate the IFN score for each sample.

Results: There was no statistically significant difference in the normalized expression of any of the six ISGs in either the rhIFN α -stimulated or unstimulated samples derived from PAXgene or Tempus tubes. The greatest difference in mean normalized expression in both unstimulated and stimulated samples was observed for *ISG15* (difference in mean normalized expression was 0.0034 and 0.11, respectively). Overall there was a strong correlation of the IFN score between PAXgene and Tempus tubes for both the unstimulated ($R^2 = 0.9117$, $p < 0.0001$) and rhIFN α -stimulated samples ($R^2 = 0.8529$, $p = 0.0001$).

Conclusion: Despite reported differences in gene expression patterns associated with samples collected in PAXgene versus Tempus tubes, our results demonstrate that 6-gene interferon scores do not differ significantly between RNA samples obtained with these two systems. These results suggest that health care and research centres can use either tubes for IFN score determination using these 6 ISGs and results can be directly compared irrelevant of the RNA collection system employed.

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