Abstract THU0004 Figure 3.

4. No effect on RAW264.7 macrophage apoptosis(Figure4)

Abstract THU0004 Figure 4.

5. Effect of miR-223 on NLRP3 inflammasome expression in RAW264.7 macrophages treated with MSU(Figure5)

Abstract THU0004 Figure 5.

Conclusion: Our findings suggest that miR-223 provides negative feedback regulation of gouty inflammation development by suppressing production of IL-1β and TNF-α, but not by regulating IL-37 and TGF-β1, and that miR-223 regulates cytokine production by targeting the NLRP3 inflammasome. These findings provide novel insight into the regulatory role of miR-223 in the spontaneous resolution of acute gouty inflammation. Finally, these results suggest that targeting miR-223 in macrophages might be an effective therapeutic strategy for the treatment of gout flare.

REFERENCES:

- [1] Henshall DC, Hamer HM, Pasterkamp RJ, Goldstein DB, Kjems J, Prehn JHM, Schorge S, Lamotte K, Rosenow F: MicroRNAs in epilepsy: pathophysiology and clinical utility. *Lancet Neurol* 2016; 15:1368-1376.

Disclosure of Interests: Yu-Feng Qing Grant/research support from: Sichuan Youth Science and Technology (2016JQ0053), and the Department of Science and Technology of Sichuan Province (2018JY0257); Dan Zhu: None declared, Ting Yi: None declared, Jian-Xiong Zheng: None declared, Qin Xiong: None declared, Quan-Bo Zhang: None declared
DOI: 10.1136/annrheumdis-2019-eular.3450

PTX3 AS A UNIVERSAL MARKER OF VASCULAR INFLAMMATION IN MULTIPLE IMMUNE-MEDIATED DISEASES

Giuseppe Alvisi Ramirez^{1,2,3}, Enrico Tombetti^{1,2,3}, Patrizia Rovere-Querini^{1,3}, Miriam Blasi^{1,3}, Mattia Baldini^{1,2,3}, Rebecca De Lorenzo^{1,3}, Enrica Bozzolo², Roberto Leone⁴, Alessandro Ambrosi¹, Alberto Mantovani^{4,5,6}, Angelo Manfredi^{1,2,3}. ¹Università Vita-Salute San Raffaele, Milan, Italy; ²IRCCS Ospedale San Raffaele, Unit of Immunology, Rheumatology, Allergy and Rare Diseases, Milan, Italy; ³IRCCS Ospedale San Raffaele, Division of Immunology, Transplantation and Infectious Immunity, Milan, Italy; ⁴Humanitas Clinical and Research Center – IRCCS, Rozzano (MI), Italy; ⁵Humanitas University, Rozzano (MI), Italy; ⁶The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

Background: Pentraxin-3 (PTX3) is a prototypic innate humoral pattern recognition receptor with a multi-functional role in tissue homeostasis from host defence to fertility, cancer biology, autoimmunity, regulation of angiogenesis and tissue repair. PTX3 is selectively expressed at sites of inflammation and rises in the circulating blood during disease activity in many (but not all) immune-mediated inflammatory conditions. Research on PTX3 as a biomarker has so far focused on single diseases.

Objectives: To identify correlations among PTX3 elevation in plasma and clinical and pathophysiological phenotypes in multiple immune-mediated diseases.

Methods: Three-hundred-sixty-six subjects encompassing 79 healthy controls and 287 patients with autoimmune diseases were enrolled. Autoimmune diseases included systemic lupus erythematosus (SLE, n=55), rheumatoid arthritis (RA, n=21), polymyalgia rheumatica (PMR, n=10), ANCA-associated vasculitides (AAV, n=63), giant cell arteritis (GCA, n=96) and Takayasu's arteritis (TA, n=42). Plasma PTX3 concentrations were measured by ELISA. Basic demographics (including gender, age at sampling and disease duration), disease activity and accrued irreversible damage, dose of prednisone or equivalents, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values at time of sampling were recorded. Disease activity and damage scores were made homogeneous by calculating Z-scores (i.e. (x-mean)/(standard deviation): Z-activity and Z-damage).

Results: Patients with SLE, AAV, TA and GCA, but not patients with RA and PMR, had higher PTX3 levels than HC (2.33 ng/ml, IQR: 1.26-4.89 vs 1.22 ng/ml, IQR: 0.80-1.98; p<0.001). PTX3 concentration correlated with Z-activity (p=0.016), ESR and CRP (p<0.001 and p=0.035 respectively) and prednisone dose (p=0.001). It was higher in females (p=0.018), in patients with disease onset within 6 months from sampling (p=0.041) and in those with previous or current active vasculitis (both p<0.001) at univariate analysis. Active small-(B=6.706, Std.Err=1.473, p<0.001), or large- vessel vasculitis (B=4.269, Std.Err=0.844, p=0.008) were the main independent variables influencing PTX3 levels at multivariate analysis.

Conclusion: High levels of PTX3 in the blood can contribute to identify an increased risk of vascular involvement in patients with systemic immune-mediated diseases.

REFERENCES:

- [1] Erreni M, et al. Immunological reviews. 2017
- [2] Ramirez GA, et al. Arthritis research & therapy. 2015
- [3] Rovere P, et al. Blood. 2000

Disclosure of Interests: Giuseppe Alvisi Ramirez: None declared, Enrico Tombetti: None declared, Patrizia Rovere-Querini Grant/research support from: Adienne Pharma & Biotech, Miriam Blasi: None declared, Mattia Baldini: None declared, Rebecca De Lorenzo Grant/research support from: Adienne Pharma & Biotech, Enrica Bozzolo: None declared, Roberto Leone: None declared, Alessandro Ambrosi: None declared, Alberto Mantovani: None declared, Angelo Manfredi: None declared

DOI: 10.1136/annrheumdis-2019-eular.7537

4. Cytokines and inflammatory mediators

THU0006

PROINFLAMMATORY RESPONSES IN THE JAK-STAT PATHWAY IN SYNOVIAL FIBROBLASTS ARE STIMULUS-SPECIFIC AND ONLY PARTIALLY INHIBITED BY THERAPEUTIC DOSES OF TOFACITINIB

Ana Županič, Blaž Burja, Kerstin Klein, Raphael Micheroli, Oliver Distler, Caroline Ospelt, Mojca Frank-Bertoncelj. University Hospital Zurich, Center of Experimental Rheumatology, Department of Rheumatology, Schlieren, Switzerland

Background: The Jak-STAT signalling pathway has a key role in the pathogenesis of rheumatoid arthritis (RA). First Jak inhibitors, including tofacitinib, have been approved for the treatment of RA. Whereas Jak inhibitors exhibit pleiotropic effects on the immune system, their "in situ" activities on synovial fibroblasts (SF), the resident synovial cells, are less well understood.

Objectives: To characterise the Jak-STAT pathway and its inhibition by tofacitinib in RA synovial tissues and SF from different joints across diverse pro-inflammatory stimuli.

Methods: Synovial tissues and SF were isolated from knee (n=4), shoulder (n=4) and hand (n=4-6) joints of RA patients undergoing joint replacement surgery. To activate and inhibit the Jak-STAT pathway, SF were stimulated with TNF (0.1 ng/ml, 1ng/ml) ± IL-6 receptor (IL-6R, 50ng/ml) or IL-6 (50ng/ml) + IL-6R for 24h in the presence or absence of 80nM, 180nM and 1000nM tofacitinib. 80nM and 180nM tofacitinib mimic the plasma drug concentrations in subjects on therapeutic doses of tofacitinib. 1000nM is the *in vitro* used tofacitinib concentration. Gene expression in synovial tissues and SF (n=12 each) was measured using the low-density gene expression arrays containing 6 housekeeper genes and 90 probes for the core components, inhibitors and target genes of

the Jak-STAT pathway. Clustering analysis of the array data [principal component analysis (PCA), heatmap] was performed using the ClustVis web tool. PCA identified one outlier SF sample, driven by distinct house-keeper gene expression that was omitted from further analysis. The production of IL-6 was determined by ELISA.

Results: RA synovial tissues and quiescent SF exhibited highly similar expression of the core components of the Jak-STAT signalling pathway, suggesting that SF contribute significantly to the synovial expression of JAK and STAT genes. Clustering analysis of the array data showed that SF stimulated with 0.1ng/ml TNF+IL-6R, SF treated with IL-6+IL-6R and quiescent SF formed separate clusters (Figure 1), pointing towards stimulus-specific transcriptional outputs in the Jak-STAT pathway. Moreover, TNF+IL-6R increased the expression of multiple core components in the Jak-STAT pathway (IL6ST, JAK2, JAK3, STAT3, STAT4) with simultaneous downregulation of pathway inhibitors (PIAS1, PIAS2) (p<0.05). SF produced different amounts of IL-6 under distinct proinflammatory stimuli, including 0.1ng/ml TNF (IL-6 mean±SD: 1.1±0.8 ng/ml), 0.1ng/ml TNF + IL-6R (2.7±1.5 ng/ml), 1ng/ml TNF (2.8±1.7 ng/ml) and 1ng/ml TNF + IL-6R (5.0±2.7 ng/ml). Specifically, the addition of IL-6R to 0.1 ng/ml TNF increased the production of IL-6 in SF to the levels observed with 1ng/ml TNF alone and this response was further exacerbated in the presence of IL-6R + 1 ng/ml TNF. 80nM, 180nM and 1000nM tofacitinib decreased the IL-6 production by 20-30%, 50% and 60% respectively, suggesting a limited anti-inflammatory effect at average plasma concentrations (80nM). The inhibitory effect of tofacitinib on IL-6 production in SF was thus dose dependent, however highly similar across distinct proinflammatory conditions (0.1ng/ml or 1ng/ml TNF ± IL-6R).

Abstract THU0006 Figure 1. Clustering analysis of the Jak-STAT transcriptional signatures in SF

Conclusion: Here we show that soluble IL-6R strongly augments the pro-inflammatory effects of TNF in SF. Furthermore, percent inhibition of the IL-6 production by tofacitinib remained constant across diverse proinflammatory conditions despite different amounts of IL-6 being produced. Thus, higher pre-treatment inflammatory responses to TNF ± IL-6R predict higher residual inflammatory activity in SF following Tofacitinib therapy, pointing towards a saturation of anti-inflammatory effects of Tofacitinib in SF in RA.

Disclosure of Interests: Ana Županič Grant/research support from: EULAR Scientific Bursary, Blaž Burja: None declared, Kerstin Klein: None declared, Raphael Micheroli: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/had consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie,