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.

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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

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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.

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THU0007 IL-26 PROMOTES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

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Background: IL-26 is a 171-amino acid protein, which is classified as a member of the Th17 cytokine family. The role of IL-26 in osteoclastogenesis in RA is needed to be clarified to understand the pathogenesis of RA.

Objectives: To examine the functional role of interleukin-26 (IL-26) in the expression of RANKL and induction of osteoclastogenesis in rheumatoid arthritis (RA).

Methods: The expression of IL-20R α , CD55 and RANKL in RA synovial fibroblasts was analyzed using confocal microscopy. RA Fibroblasts like synoviocytes (RA-FLS) were treated with recombinant human IL-26, and the expression of RANKL messenger RNA (mRNA) and protein was measured using real-time polymerase chain reaction and ELISA. Human peripheral blood monocytes were cultured with macrophage colony-stimulating factor and IL-26, after which osteoclastogenesis was evaluated by counting the number of tartrate-resistant acid phosphatase-positive multinucleated cells. Osteoclastogenesis was also evaluated after monocytes were co-cultured with IL-26-prestimulated FLS.

Results: The IL-26 concentration in the FLS was higher in RA patients than in patients with osteoarthritis (OA). In RA-FLS treated with rhIL-26, the expression of RANKL mRNA and protein was increased in a dose-dependent manner. IL-26 increased the expression of RANKL in RA-FLS, and the IL-26-induced RANKL expression decreased by the inhibition of IL-20R α . IL-26-induced RANKL expression was down-regulated significantly by the inhibition of SHP-1, ERK, JNK, stat1, c-jun or p38 MAPK/NF- κ B signaling. When monocytes isolated from human peripheral blood were cultured with IL-26, they were differentiated into osteoclasts in the absence of RANKL. Monocytes were also differentiated into osteoclasts when they were cocultured with IL-26-pre-treated RA-FLS.

Conclusion: IL-26 has a dual effect on osteoclastogenesis in RA: 1) direct induction of osteoclastogenesis from monocytes and 2) up-regulation of RANKL production in RA-FLS. This IL-26/RANKL axis could be a potential therapeutic target for bone destruction in RA.

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THU0008 GM-CSF PATHWAY SIGNATURE IDENTIFIED IN TEMPORAL ARTERY BIOPSIES OF PATIENTS WITH GIANT CELL ARTERITIS

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Background: Giant Cell Arteritis (GCA) is a type of large vessel vasculitis that can cause blindness and aortic aneurysms. A significant unmet medical need remains in GCA, as current treatment options are limited, and relapse increases corticosteroid (CS) exposure and toxicity.

The primary role of macrophages/dendritic cells (DCs) and T_H1/T_H17 lymphocytes in GCA pathogenesis has been highlighted previously. Granulocyte-macrophage colony stimulating factor (GM-CSF) may contribute to GCA pathogenesis by stimulating giant cell formation.¹ GM-CSF produced by CD4⁺ T helper T_H1 and T_H17 cells can stimulate conventional DCs

and promote differentiation of monocyte-derived DCs.² GM-CSF may drive DCs to program naïve CD4⁺ cells to T_H1, T_H17, and T follicular helper phenotypes (IFN γ /IL-17/IL-21). Notably GM-CSF RNA has been reported in GCA lesions³ and in peripheral blood mononuclear cells of symptomatic patients.⁴

Objectives: We hypothesized elevation of the GM-CSF pathway signature in GCA vessels versus controls.

Methods: Two independent sources of temporal artery biopsies were utilized. First, GCA (n=17) and control (symptomatic patients suspected for GCA, but with a normal temporal artery biopsy; n=5) biopsies were analyzed for 15 mRNA transcripts representing T_H1, T_H17, and GM-CSF signaling (RNAscope; RS) and for mRNA transcripts representing the autoimmune panel (Nanostring; NS). Semi-quantitative scoring was performed on RS images, and fold-change of representative T_H1, T_H17 and GM-CSF related mRNA transcripts were calculated via NS nCounter analysis. Additional GCA and control biopsies were obtained and analyzed by RT-PCR for a subset of transcripts (n=10 each) and by confocal microscopy for GM-CSF and GM-CSF-R α protein (n=2 each).

Results: The GM-CSF signaling pathway molecular signature was confirmed to be upregulated by 4 independent analyses.

GM-CSF-associated and T_H1-associated genes were upregulated in GCA biopsies versus control (GM-CSF: 3-4x RS; GM-CSF-R α : 6.7x NS, 6x RS; and CD83: 3.9x NS, 6x RS; TNF α : 2x NS, 3x RS; IFN γ : 2x RS; IL-1 β : 6x RS). T_H17 associated genes were not elevated, potentially due to concomitant CS treatment.

Upregulation of both GM-CSF (12x) and GM-CSF-R α (3x) mRNA was confirmed in a separate cohort of biopsies from GCA patients vs. controls by RT-PCR (Figure). GM-CSF and GM-CSF-R α proteins were detected in the luminal endothelium, neovessels and inflammatory cells of GCA patients. In normal temporal arteries, GM-CSF protein was not detected, and some GM-CSF-R α expression was observed in the luminal endothelium.

Pu.1, a transcription factor downstream of GM-CSF signaling, was increased 8x in GCA vs. controls (RS, NS) (Figure).

Conclusion: GM-CSF and T_H1 pathway signatures were demonstrated in GCA patient temporal arteries by independent analytical techniques. Active GM-CSF signaling in diseased tissue is evidenced by increased expression of Pu.1 in the vessel wall. These data implicate the GM-CSF pathway in GCA pathophysiology and increase confidence in rationale for targeting GM-CSF in GCA.

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Abstract THU0008 Figure 1.