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 TOFACTINIB**

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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 vitro 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.

**Results:** FACS analysis of cytokine expression in synovial tissues and SF (n=12 each) was measured using the low-density gene expression arrays containing 6 housekeeper genes 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).

**Conclusion:** The inhibitory effect of tofacitinib on 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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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, 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 tartrateresistant acid phosphatasepositive multinucleated cells. Osteoclastogenesis was also evaluated after monocytes were co-cultured with IL-26-prestimulated FLS.

Results: The IL-26 expression in the FLS was higher in RA patients than in patients with osteoarthritis (OA). In RA-FLS treated with IL-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. IL-26–induced RANKL expression was down-regulated significantly by the inhibition of SHP-1, ERK, JNK, stat1, c-jun or p38 MAPK/ NF-κB 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–prestimated 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.

REFERENCE:

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THU0007

GM-CSF PATHWAY SIGNATURE IDENTIFIED IN TEMPORAL ARTERY BIOPSY SPECIMENS 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_{h}1/T_{h}17 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. GM-CSF may drive DCs to program naïve CD4+ T cells to T_{h}1, T_{h}17, and T follicular helper phenotypes (IFNγ/IL-17/L-21). Notably GM-CSF RNA has been reported in GCA lesions and in peripheral blood mononuclear cells of symptomatic patients.

Objectives: We hypothesized elevation of the GM-CSF pathway signature in GCA 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 temporary artery biopsy; n=5) biopsies were analyzed for 15 mRNA transcripts representing T_{h}1, T_{h}17, 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_{h}1, T_{h}17 and GM-CSF related mRNA transcripts were calculated via NS nCounter analysis. Additional GCA and control biopsy specimens 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-Rx 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_{h}1-associated genes were upregulated in GCA biopsies versus control (GMCSF: 3.4x RS; GM-CSF-Rx: 6.7x NS, 6x RS; and CD83: 3.9x NS, 6x RS; TNFx: 2x NS, 3x RS; IFNγ: 2x RS; IL-1β: 6x RS). T_{h}17 associated genes were not elevated, potentially due to concomitant CS treatment.

Upregulation of both GM-CSF (12x) and GM-CSF-Rx (3x) mRNA was confirmed to be in a separate cohort of biopsies from GCA patients vs. controls by RT-PCR (Figure). GM-CSF and GM-CSF-Rx 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-Rx expression was observed in the luminal endothelium.

Pun1, a transcription factor downstream of GM-CSF signaling, was increased 8x in GCA vs. controls (RS, NS) (Figure).

Conclusion: GM-CSF and T_{h}1 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 Pun1 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.

REFERENCES: