Background: Interleukin-1 beta (IL-1b) is a key mediator of the inflammatory response and is known to elicit damage during chronic disease and acute tissue injury. Through association with the adaptor protein Myd88, interleukin receptor associated kinases (IRAK)1 and 4 initiate signaling downstream of IL-1Rs resulting in the activation of the NFkB and MAPK pathways and the production of proinflammatory cytokines (1). IL-1Rs are broadly expressed across cell types and little is known about differences in signaling between cell types and the role of IRAK1 and IRAK4 kinase activity.

Objectives: We have identified a potent and selective IRAK1/4 inhibitor, R835, that substantially suppressed the elevation of LPS (TLR4 agonist)-induced serum cytokines in healthy human volunteers in a recent phase 1 study. The aim of this study was to evaluate the effect of R835 on IL-1R signaling in primary human fibroblasts and endothelial cells.

Methods: Human dermal fibroblasts, lung fibroblasts or endothelial cells were stimulated with IL-1b and the effect of R835 on the signaling pathway was evaluated by western blotting. Human dermal fibroblasts were stimulated with different amounts of IL-1b to evaluate both the signaling pathways activated and the cytokines produced. The ability of R835 to inhibit cytokine production induced by high or low amounts of IL-1b in dermal fibroblasts was assessed.

Results: In human endothelial cells, inhibition of IRAK1/4 kinases with R835 resulted in a block of IL-1b-induced IRAK4 phosphorylation, IRAK1 degradation and downstream NFkB, p38 and JNK activation. In contrast, in both human primary dermal and lung fibroblasts stimulated with IL-1b, we observed potent inhibition of IRAK4 phosphorylation, IRAK1 degradation, and downstream JNK phosphorylation, but no inhibition of NFkB pathway proteins and only weak inhibition of p38. Upon titration of IL-1b we observed that dexamethasone produced IL-8 and GRO in response to low levels of IL-1b (20pg/ml), and produced additional cytokines including G-CSF and GM-CSF with higher levels of IL-1b (400pg/ml). In the presence of low levels of IL-1b (20pg/ml), we observed a weak activation of NFkB pathway proteins and p38, compared to a very robust NFkB, p38 and additional JNK activation in the presence of higher levels of IL-1b (400pg/ml). Consistent with these results, in dermal fibroblasts, R835 showed little to no inhibition of IL-8 and GRO induced by low levels of IL-1b, but potently inhibited G-CSF and GM-CSF induced by high levels of IL-1b where JNK was activated.

Conclusion: This study has elucidated signaling differences between cell types downstream of the IL-1R. In endothelial cells, as in myeloid cells, the kinase activity of IRAK1 and IRAK4 is required for the activation of all downstream signaling. Unexpectedly, in human fibroblasts, IRAK1/4 kinase activity appears to primarily regulate the JNK pathway, and not the NFkB pathway. Combinant with that, only the cytokines induced by the additional activation of JNK in fibroblasts are regulated by a dual IRAK1/4 inhibitor. Clinically, an IRAK1/4 inhibitor may show select inhibition of IL-1b-induced cytokines depending on the tissue and cell type involved in inflammation.

Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2020-eular.3852
Background: STING (stimulator of interferon genes) is a cytosolic protein that is found in endosomal reticulum (ER) membrane, mitochondria and mitochondria-associated membranes. Although it is well established that STING plays an important role in innate immune responses, its potential involvement in rheumatic disease processes remains to be clarified.

Objectives: The aims of this study were to evaluate the levels of STING and its relationship with local inflammation in the synovial fluid (SF) of patients with psoriatic arthritis (PsA), rheumatoid arthritis (RA), gout, calcium pyrophosphate (CPP) crystal-induced arthritis (CPP-IA), osteoarthritis (OA) and OA with CPP crystals (OA+CPP).

Methods: SF was collected from the knees of 60 untreated patients: 10 with PsA, 10 with RA, 10 with gout, 10 with CPP-IA, 10 with OA and 10 with OA+CPP. SF was examined under optical light microscopy. White cell count (WBC) and the polymorphonuclear cell (PMN) percentage were determined in SF according to standard procedures. SF IL-6, IL-8, IL-1β and extra- and intra-cellular STING levels were assessed by ELISA.

Results: The levels of WBC were higher in SFs from gouty patients (27.7±2.05 10³/mm³), while OA and OA+CPP patients showed the lowest WBC count (0.3±0.0 10³/mm³, 0.3±0.28 10³/mm³). SFs from inflammatory arthritis contained elevated percentages of PMN (gout: 85.5±10.86%, CPP-IA: 84.3±17.11%, RA: 80.33±18.14%, PsA: 42.6±13.97%). Extracellular STING was determined in OA (440±131.31 pg/ml), OA+CPP (225±205.06 pg/ml) and CPP-IA (475±707 pg/ml). SF, while not detectable in RA, PsA and gout. Intracellular STING levels were similar and the highest in SFs from gout (96.4±35.44 pg/ml) and RA (90.6±23.13 pg/ml). While remaining under detect limit only in SFs from PsA, SF concentration of IL-6 was lower in OA (354.8±1077.56 pg/ml) and OA+CPP (389.56±104.14 pg/ml) as compared with inflammatory arthritis SFs (PsA: 3807±1448.86 pg/ml; RA: 17354±2334.87 pg/ml; gout: 19359±848.5 pg/ml; CPP-IA: 20389.56±104.14 pg/ml). The patients with gout and RA had the highest levels of IL-8 (2159.5±3470.9 pg/ml; 2039.6±977.4 pg/ml) and IL-1β (35.93±20.46 pg/ml; 44.36±23.16 pg/ml). While OA showed the lowest concentrations (IL-8: 23.21±11.32 pg/ml; IL-1β: 0.47±0.13 pg/ml). In the total group of patients, we found a negative correlation between extracellular STING and IL-8 (r=-0.53, p=0.004) and IL-1β (r=-0.47, p=0.012). There was a positive correlation between intracellular STING and IL-6 (r=0.54, p=0.017), IL-1β (r=0.77, p<0.001) and IL-6 (r=0.69, p=0.009).

Conclusion: This study is the first to define the presence of STING in SF of different arthritides. The high levels of intracellular STING in OA, OA+CPP and CPP-IA SFs may be due to the activation of factors that reduce its interaction with the ER. The effect of downregulating factors in PsA might explain the low concentration of intracellular STING in these patients.


Disclosure of Interests: None declared.

Acknowledgements: Project MH CR 00023728 & MEYS CR Progres Q43 Disclosure of Interests: Patrik Škubica: None declared, Jana Horinkova: None declared, Monika Gregović Consultant of: Novartis, Abbvie, Pfizer; Principal investigator for: Novartis, Speakers bureau: Novartis, Abbvie, MSD, Karel Pavěka Consultant of: Abbvie, MSD, BMS, Esis, Roche, UCB, Medac, Pfizer, Biogen, Speakers bureau: Abbvie, MSD, BMS, Elys, Roche, UCB, Medac, Pfizer, Biogen, Martka Husakova Speakers bureau: Novartis, Pavlina Dankova: None declared DOI: 10.1136/annrheumdis-2020-eular.7774

AB0062
CHARACTERIZATION OF IL-12 AND IL-23 REDUCTION BY TOFACITINIB IN MDCS
N. Vincen1, C. Angiolilli2, S. Cardoso3, A. Lopes4, M. Olde-Nordkamp5, R. Radstake5, 1Center of Translational Immunology, Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, Netherlands

Background: Psoriatic arthritis (PsA) is a chronic inflammatory auto-immune disease characterized by an excessive production of pathogenic mediators that cause inflammation of the skin, peripheral joints, entheses and the spine. Among these, interleukin (IL)-23, IL-12, the IL-17 family and TNF contribute key players in PsA pathogenesis.1-2 IL-23, consisting of IL23A (IL-23p19) and IL12B (IL-12p40) subunits, is predominantly produced by myeloid dendritic cells (mDCs). While the p19 subunit is unique to IL-23, the p40 subunit is shared with IL-12. Together, IL-12 and IL-23 play a crucial role in promoting the differentiation of naïve T lymphocytes into Th1 helper (Th1) and Th17 helper (Th17) cells, respectively.3-4 Small-molecule inhibitors, such as the JAK/STAT inhibitor Tofacitinib, have recently shown promising therapeutic potential in PsA clinical trials.3-5 The inhibition of JAKs by Tofacitinib results in the direct suppression of multiple intracellular signaling pathways which constitute key hubs in the cytokine network.6 However, whether Tofacitinib is able directly target IL-12/IL-23 production by mDCs has not yet been documented. Suppression of these canonical inflammatory pathways would provide further evidence that Tofacitinib is an effective drug in halting both innate and adaptive immune responses.

Objectives: To evaluate the transcriptional and molecular events underlying IL-12 and IL-23 regulation by Tofacitinib in mDCs.

Methods: Peripheral blood mononuclear cells from healthy donors were isolated by Ficoll gradient. Monocytes and myeloid dendritic cells (mDCs) were isolated by using magnetic beads on autoMACS. Monocytes were cultured for 6 days in the presence of IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) to generate monocyte-derived dendritic cells (mDCs). mDCs were harvested, washed and put to rest for 1 day prior to stimulation, while mDCs