Results: Participants had a mean age 47 ± 9 yrs and 6 out of 7 (86%) were female. All had KL grade ≥ 2 with substantial knee pain at baseline. Enough SF (>500 μ l) could be obtained from $n=7$ at baseline, $n=4$ at midpoint, and $n=3$ at endpoint of distraction. For the first time in this same group, we show how MSC number initially decline upon KJD (figure 1A-B) as seen previously in our animal study [4]. Also, MSCs present in the SF showed changes in their gene expression profile upon KJD, most clearly observed during the treatment (3 weeks; figure 1C). GDF5 and Grem1 presented with a statistically significant increased expression ($p < 0.05$) during treatment while FAB4 expression was decreased. ACAN, PTH1R, and DDR expression had the tendency to increase over time. ADAMTS4, SOX9 and PTHLH expression showed a trend to decrease over time.

Conclusion: This explorative study provides for the first-time data on changes in SF MSC number and their gene expression profiles upon knee joint distraction. As such, first clues are provided for the involvement of MSCs in the regenerative process induced by joint distraction for end-stage knee OA. The fall in SF MSCs number during distraction suggests adhesion to the arthritic surfaces in the KJD environment as found in our previous work in canine KJD [4]. Further studies are necessary to unravel the processes involved.

REFERENCES:

- [1] SM Kurtz, et al. Clin Orthop Relat Res 2009, 467(10):2606-
- [2] J-TAD van der Woude, et al. Cartilage 2017, 8(3):263-
- [3] MP Jansen, et al. Osteoarthritis Cartilage. 2018; 26(12):1604-
- [4] T Baboolol, et al. Ann Rheum Dis. 2016;75(5):908-

Acknowledgement: Funded by FOREUM and Dutch Arthritis Society.

Disclosure of Interests: Thomas Baboolol: None declared, Mylène Jansen: None declared, Elena Jones: None declared, Carla Sanjurjo-Rodríguez: None declared, Tim Welting: None declared, Pieter Emans: None declared, Floris Lafaber Shareholder of: ArthroSave, Grant/research support from: FOREUM; Dutch Arthritis Society, Dennis McGonagle Consultant for: Lilly, Novartis UCB, Speakers bureau: Lilly, Novartis UCB, Simon Mastbergen Grant/research support from: FOREUM; Dutch Arthritis Society
DOI: 10.1136/annrheumdis-2019-eular.3001

Figure 1. Changes in synovial fluid MSCs numbers and gene expressing profiles upon knee joint distraction. Bar indicates statistically significant changes ($p < 0.05$).

FRI0519

IDENTIFICATION OF CELLULAR TARGETS FOR ANTI-CITRULLINATED PROTEIN ANTIBODIES (ACPAS)

Bence Réthi, Meng Sun, Alexandra Circumaru, Akilan Krishnamurthy, Heidi Wähämaa, Jimmy Ytterberg, Vivianne Malmström, Lars Klareskog, Anca Catrina. Karolinska Institutet and Karolinska University Hospital, Department of Medicine, Solna, Stockholm, Sweden

Background: Autoantibodies against citrullinated proteins (ACPAs) have been suggested to play important roles in RA pathogenesis (1-3).

Objectives: In order to further dissect how ACPAs influence bone homeostasis and inflammation in the joints we analyzed the binding of different ACPA clones to target antigens produced by synovial fibroblasts, osteoclasts (OCs) or neutrophil granulocytes.

Methods: Fibroblasts were isolated from synovial tissue of RA patients by enzymatic digestion. Monocytes and neutrophil granulocytes were isolated from the peripheral blood of healthy individuals. Osteoclasts were generated from monocyte-derived dendritic cells in the presence of M-CSF and RANK-L. Polyclonal ACPAs were purified from pooled serum samples of RA patients using protein G and Cyclic Citrullinated Peptide-based affinity chromatography. Monoclonal ACPAs were isolated from synovial plasma cells of RA patients, as described (4). Protein citrullination was analyzed with mass spectrometry. ACPA binding was analyzed using confocal microscopy and flow cytometry. Neutrophil extracellular trap (NET) formation was monitored using IncuCyte and confocal microscopy.

Results: Synovial fibroblasts expressed surface antigens to the ACPA clone 1325:01B09 following exposure to cellular stress (serum withdrawal) or IL-8 stimulation. Osteoclasts and their precursors were characterized by steady state protein citrullination and these cells were recognized by another distinct ACPA clone 1325:04C03. Using mass spectrometry, we detected citrullinated actin and vimentin in developing OCs. Interestingly, both proteins were present on the cell surface, showing a partial colocalization with the cell-bound 1325:04C03 antibodies.

Neutrophil granulocytes exposed a large amount of citrullinated targets for both 1325:04C03 and 1325:01B09 as well as for polyclonal ACPAs in neutrophil extracellular traps (NETs), irrespectively of the stimulus that triggered NETosis. Activation of the cells by Ca^{2+} ionophores triggered a rapid NETosis and cellular disintegration, in parallel with a profound reactivity of all tested ACPAs with both intracellular or surface antigens. Other activation signals, e.g. PMA, nigericin, zymosan or IL-8 did not induce protein citrullination in the cells, even in conditions where citrullinated proteins were detected in NETs.

Conclusion: Distinct monoclonal ACPAs target different cell types, suggesting unique pathological roles for individual ACPA clones. OCs might be among the earliest ACPA targets due to their physiological protein citrullination. Fibroblasts, on the contrary, acquired ACPA sensitivity only following cellular stress signals. Intact neutrophils were not targeted by ACPAs, whereas damaged cells and the extruded NET products might be important sources of citrullinated antigens that form immune complexes with ACPAs.

REFERENCES:

- [1] Harre, U. et al. J Clin Invest (2012)
- [2] Krishnamurthy, A. et al. Ann Rheum Dis (2016)
- [3] Wigerblad, G. et al. Ann Rheum Dis (2016)
- [4] Steen, J. et al. Arthritis Rheumatol (2018)

Disclosure of Interests: Bence Réthi: None declared, Meng Sun Grant/research support from: Yes, but not for presented project., Alexandra Circumaru: None declared, Akilan Krishnamurthy: None declared, Heidi Wähämaa: None declared, Jimmy Ytterberg Employee of: Currently employed by Sobi, Stockholm, Sweden. Earlier, during his contribution in the presented study he was 100% employed by the Karolinska Institutet., Vivianne Malmström Grant/research support from: Yes, but not for the presented study., Lars Klareskog Grant/research support from: Yes, but not for the presented study., Anca Catrina Grant/research support from: Yes, but not for the presented study.

DOI: 10.1136/annrheumdis-2019-eular.4179