expression of MMP1 and MMP3 (SF1), 2) THY1hi CD34+ fibroblasts expressing high levels of P16 (SF2), 3) THY1hi fibroblasts expressing high levels of peristin (POSTN) and collagens (e.g. COL1A1, COL3A1) (SF3), 4) THY1hi fibroblasts expressing CXCL12 (SF4), and 5) THY1hi fibroblasts expressing CXCL12, NR4A1 and CCL2 (SF5). Fig. 2 shows pathway enrichment map of all marker genes; it organizes enriched terms into a network with edges connecting overlapping gene sets. Pseudotime trajectory axis derived from Monocle indicated that SF4 represent a state between SF3 and SF5. Pseudotemporal expression dynamics of THY1 marked the progression of these three subtypes (Graph 1). SF1 and SF2 were proportionally underrepresented and SF3-5 overrepresented in RA (chi-squared = 37.18, p = 1.65e-07). The expression of POSTN, a signature gene of SF3, was not different between RA and OA tissues, but significantly correlated with the synovitis score (Spearman ρ = 0.55, p=0.02), in particular with pathological changes in the sublining. POSTN expression was higher in hand than in knee synovial tissues (mean ± SD IHC score: hand 8 ±2, knee 5 ±2) and in cultured SF (qPCR: 10-fold difference). Accordingly, SF3 was enriched in hand versus knee synovial tissues in the scRNA-seq dataset (chi-squared = 944.87, p < 2.2e-16).

Conclusion: In our meta-analysis, we found comparable subtypes of fibroblasts as in the individual analyses [1-3], showing the robustness of cell phenotype identification using scRNA-seq. The different SF phenotypes appear to be plastic cell states rather than fixed cell subtypes, whose development is controlled by an interrelation between pathological changes in the synovium and joint location.

References:

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OP0243 SERPIN3A3 LIMITS CARTILAGE DESTRUCTION IN OSTEARTHRITIS BY INHIBITING MACROPHAGE-DERIVED LEUKOCYTE ELASTASE

A. Latourte1,2, A. Combier3, S. Jaulerry1, C. Cherifi1, Y. Jouan1, H. K. E1,2, M. Cohen Solal1,2, E. Hay4, P. Richefe1,2,1Hopital Lariboisiere, Rheumatology, Paris, France; 2Hopital Lariboisiere, U1132, Paris, France

Background: Interleukin-6 (IL-6) plays an important role in osteoarthritis (OA). Transcriptomic analyses (RNAseq) revealed that SerpinA3N, a serine protease inhibitor, is a key target of IL-6 in chondrocyte.

Objectives: This study aimed to examine the role of SerpinA3N and Leukocyte Elastase (Elane), a serine protease targeted by SerpinA3N, in cartilage destruction during OA.

Methods: The role of SerpinA3N was investigated in the destabilization of medial meniscus (DMM) model of murine OA with 1) mice with conditional inducible knockdown of Serpina3n in cartilage (Col2CreER.Serpina3nfl/fl mice [A.Serpina3nfl/fl]) and 2) C57BL/6 wild type (WT) mice treated with intra-articular injection of SerpinA3N (1.5 or 15mM/week). OA joint lesions were assessed by histology (OARSI and synovitis scores) and micro-CT analysis (osteophyte volume, subchondral bone remodelling).

Because serine proteases targeted by SerpinA3N are not produced by murine chondrocytes, Elane expression (qRT-PCR) was determined in murine macrophages (Raw) stimulated or not by IL-6 (100 ng/ml). Recombinant

Fig. 1

Graph 1

Fig. 2
SerpinA3N (30 nM) and a specific Elane inhibitor, Sivelestat (100 µg/ml) were used on cartilage explants treated by conditioned medium of macrophages pre-treated or not by IL-6 (CM–IL-6). Cartilage catabolism was determined by histology and matrix metalloproteinase MMP-3 production was evaluated by Western Blot and immunohistochemistry (IHC). Weekly intra-articular injections of Sivelestat (1mM) were performed in the DMM to determine the role of Elane in OA.

Results: ΔSerpinA3N ΔTice mice had more severe OA lesions than control littermates 6 weeks after DMM, with greater cartilage damage (mean±SD OARSI score: 5.6±0.4 vs 4.4±0.3, p=0.01), increased synovitis scores (3.0±0.3 vs 1.9±0.3, p=0.03) and bigger osteophytes (7.2±0.8x107 vs 3.8±0.8x107, p=0.048). Conversely, WT mice treated with intra-articular injections of SerpinA3N 15nM exhibited less severe cartilage loss than mice treated with PBS after DMM (OARSI score: 2.1±0.4 vs 3.9±0.5, p=0.02). Elane mRNA expression was increased in macrophages upon IL-6 stimulation. In cartilage explants, CM–IL-6 activated cartilage catabolism and MMP-3 production, and effect that was blunted by SerpinA3N and Sivelestat. Finally, mice treated with intra-articular injections of Sivelestat had less severe cartilage damage than those treated with PBS after DMM (OARSI score: 3.3±0.47 vs 5.8±0.53, p=0.0046).

Conclusion: SerpinA3N protects against experimental OA via the inhibition of Elane, a pro-catabolic serine protease produced by macrophages. This results highlight the crosstalk between cartilage and surrounding macrophages and open up new therapeutic perspectives.

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OP0245 MICROBIOTA-INDUCED INTESTINAL BARRIER DYSFUNCTION PRECEDES THE ONSET OF ARTHRITIS AND ALLOWS THE SHUTTLING OF IMMUNE CELLS FROM THE GUT TO THE JOINTS

M. Zais1, N. Tajic1, K. Sarter1, V. Azizov1, L. Bucci2, Y. Luo3, J. D. D. Cañete4, F. Ciccia5, G. Schett1, 1FAU Erlangen, Erlangen, Germany; 2Università degli Studi della Campania, Napoli, Italy; 3Sichuan University, Chengdu, China; 4Università degli Studi della Campania, Napoli, Italy; 5Department of Precision Medicine, Erlangen, Italy

Background: While it is known that microbial dysbiosis is associated with the onset of neutrophilic arthritis, mechanistic insights into how it facilitates the development of arthritis remained largely elusive to date. It is especially interesting how microbial dysbiosis affects the transition from asymptomatic autoimmunity to arthritis. We speculated that a breakdown of intestinal barrier function caused by microbial dysbiosis allows immune cells to shuttle from the gut to the joints.

Objectives: To test whether intestinal barrier function is impaired before the onset of human RA and experimental arthritis and to seek for evidence that intestinal immune cells migrate to the joints.

Methods: In a longitudinal cohort of RA-at-risk individuals markers of disturbed intestinal barrier function, such as zonulin, were analysed and linked to RA onset. Furthermore, new-onset RA patients were assessed for gut leakiness and their intestinal biopsies for the expression of tight junction proteins and immune cell infiltration. In the murine model of collagen-induced arthritis, sequential analysis of intestinal dysbiosis, intestinal barrier function and arthritis onset was carried out. Additionally, barrier function was assessed on intestinal organoids exposed to faecal supernatants from eu- and dysbiotic mice with and without inhibition of zonulin. Furthermore, three types of interventions restoring intestinal barrier function were carried out for testing their effects on the inhibition of arthritis onset. Finally, photo-converted cells from the gut were traced in the joints to test for the possibility of trafficking from the gut to the joints.

Results: Zonulin, a potent regulator for intestinal tight junctions, was elevated in autoimmune mice and men before the onset of arthritis and predicted the onset of human RA. Intestinal barrier functions as well as epithelial tight junctions were decreased before the onset of experimental arthritis and at onset of human RA. In mice, induction of autoimmunity was followed by rapid intestinal dysbiosis followed by gut leakiness before arthritis started. Faecal supernatants of arthritic mice induce epithelial barrier dysfunction in intestinal organoids in zonulin dependent manner. Restoration of the intestinal barrier in the pre-phase of arthritis using butyrate, CB1R agonist or zonulin antagonist larazotide inhibited the development of arthritis. Finally, using photoconvertible mice, gut-borne immune cells were identified that homed to the joints when barrier function was impaired.

Conclusion: In summary, these data show the intestinal barrier dysfunction precedes the onset of RA and allows the trafficking of immune cells from the gut to the joints. Targeting of intestinal tight junction function may therefore allow preventing the onset of RA.

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