Conclusion: Our results show that Malt1 seems to be an important molecule in the development and progression of experimental autoantibody-induced arthritis in mice, highlighting the role of the molecule as a potential therapeutic target in the future.

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AB0099

METHOTREXATE REDUCES THE INVASIVE ACTIVITIES OF PRIMARY RA SYNOVIAL FIBROBLASTS

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Background: Rheumatoid Arthritis synovial fibroblasts (RASFs) are key player in tissue destruction via the production of a wide range of chemical reactions in the joint with high growth rate and resistance to mortality [1]. Methotrexate (MTX) is a dihydrofolate reductase inhibitor that attenuates inflammation within joints resulting in reduced cartilage and bone damage and is the anchor therapy for RA. Its mechanisms of action are thought to differ from its anti-proliferative effects and are known to include increased adenosine release (2), but may also involve alterations in intracellular methyl donor status resulting in alteration in DNA methylation and gene expression.

Objectives: To investigate the effects of MTX on RASFs auto-aggressive activities, including invasion, migration, proliferation and apoptosis.

Methods: RASF were derived from knee biopsies of RA patients taken at arthroscopy (n=9). Matrigel chambers were used to measure invasive activities. The cells were incubated with DMSO (control), 1µM or 10µM MTX for 96 hours. Wound healing (scratch assays) were used to measure migration. Proliferation and apoptosis was determined using BrdU and caspase-3/7 assays respectively. Significance was determined via repeated measures ANOVA using SPSS software.

Results: Incubation with MTX resulted in significantly reduced invasive activity compared with DMSO control; 1µM (35%, p=0.006) and 10µM (58%, p=0.002) in paired samples. However MTX did not have significant effects on RASF migration, apoptosis or proliferation at either concentration.

Conclusion: Our data reveals that MTX reduces the invasive potential of RASFs in vitro, this effect may contribute to the clinical efficacy of this agent. Further investigation will involve epigenome-wide methylation to determine if the DNA methylome of RASFs is altered by MTX.

References:

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AB0100

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF SYNOVIAL FLUID-DERIVED FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Background: Fibroblast-like synoviocytes (FLS) are central cellular components in persistent inflammatory joint diseases such as

rheumatoid arthritis (RA). Pathological subsets of FLS have been identified from synovial tissue. However, the synovial tissue obtained from arthroplasty procedures is acquired at late disease stages and the cellular yield obtained from synovial tissue biopsies is fairly low. Collectively, challenging the robustness of human RA *in vivo* and *in vitro* models. FLS obtained from the synovial fluid (SF-FLS) are proposed as an alternative source of FLS, but a detailed phenotypical and functional characterization of FLS subsets from the synovial fluid has not been performed.

Objectives: The aim of this study was to determine the phenotypical and functional characteristics of synovial fluid-derived fibroblast-like synoviocytes in rheumatoid arthritis.

Methods: In the present study, paired peripheral blood mononuclear cells (PBMC) and SF-FLS from patients with RA were obtained (n=7). FLS were isolated from the synovial fluid by a strict trypsinization protocol¹ and their cellular characteristics and functionality were evaluated at passage 4. Monocultures (SF-FLS) and autologous co-cultures (SF-FLS and PBMC) were established from five patients with RA and subsequently evaluated by flow cytometry, Western blotting and multiplex immunoassays. Human cartilage-sponges (n=3) with SF-FLS and without SF-FLS (n=3) were co-implanted subcutaneously in SCID mice (n=15), mice with only cell-free human cartilage-sponges were used as controls (n=12). After 45 days, the implants were evaluated using stained sections to determine the SF-FLS invasion score based on perichondrocytic cartilage degradation. Data are expressed as median (25-75 percentile). P-values <0.05 were considered statistically significant.

Results: The homogeneous subpopulations of FLS, isolated from the synovial fluid, were negative for CD34 and CD45 [98.9%, (97.5-99.7)] and positive for Thy-1 and PDPN [94.6%, (79.9-97.4)]. Without stimulation, RA SF-FLS showed high and comparable levels of NFκB related pathway proteins and secreted multiple pro-inflammatory cytokines and chemokines dominated by IL-6 [2648 pg/mL, (1327-6116)] and MCP-1 [2458 pg/mL, (692-8719)]. SF-FLS increased their ICAM-1 and HLA-DR expression after encountering autologous PBMCs (p<0.01), (p<0.05). Further, SF-FLS and PBMC interacted synergistically in a co-culture model of RA and significantly increasing the secretion of several cytokines (IL-1β, IL-2, IL-6, (p<0.01)) and a chemokine (MCP-1, (p<0.01)). The invasion score of the human SF-FLS *in vivo* was at primary site, [1.6, (1.3-1.7)] and contralateral implantation site [1.5, (1.1-2.2)]. The invasion score of the human SF-FLS-containing implants both at primary and contralateral site were significantly higher compared with cartilage-sponges evaluated from SF-FLS-free control mice (p<0001).

Conclusion: This phenotypical and functional characterization of SF-FLS, acquired and activated at the site of pathology, lays a foundation for establishing *in vivo* and *in vitro* FLS models. These FLS models will be beneficial in our understanding of the role of this cellular subset in arthritis and for characterization of drugs specifically targeting this pathological RA FLS subset.

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