Background: Sphingosine-1-phosphate (S1P) is a biologically active phospholipid, which is derived from membrane lipid. It binds to the receptors, named S1P1–5, and regulates several signalling pathways involved in inflammation, cell survival, angiogenesis and cell migration. Concentration of S1P and expression of S1P receptors can vary according to local tissue conditions. RA is a chronic inflammatory disorder of joints and the concentration of S1P in synovial fluid is higher in RA patient than in OA patient. In vitro, S1P3 expression in RA synoviocytes is upregulated by TNFa treatments. On the other hand, it is not clariﬁed whether S1P/S1P signaling pathway contributes to arthritis in RA patients.

Objectives: The objective of this study is to investigate the role of S1P/S1P signaling in inﬂammatory arthritis.

Methods: Collagen-induced arthritis (CIA) was induced by subcutaneous injection of bovine type II collagen emulsified in complete Freund’s adjuvant in wildtype (WT) or S1P3-knock-out (S1P3-KO) 7–9 week-old DBA/1J mice. Arthritis severity were evaluated by visual scoring and histological analysis. The severity was assessed over time by using the arthritis score, in which each paw was scored on a scale of 0–4 and the scores of all four paws were cumulated, resulting in a maximum possible score of 16 per mouse. For histopathological examination, mice were sacriﬁced on the 42nd day and the hindlimbs were removed and ﬁxed in 4% buffered formaldehyde. Paraffin embedded sections of the knee joints stained with hematoxylin and eosin were systematically scanned in a microscope and scored based on cell inﬁltration, cartilage destruction and bone erosion parameters. S1P3 mRNA expression was examined by real-time PCR method with total RNA extracted from knee joint capsules of CIA or normal WT mice. Murine primary ﬁbroblast like synoviocytes (FLS) were obtained from CIA mice. We examined S1P3 expression after TNFa treatment and measured cytokine production after S1P treatment with or without TNFa pretreatment in FLS.

Results: S1P3 deﬁciency resulted in modest symptoms of arthritis and a signiﬁcant reduction in synovial inﬂammation and bone erosions in histological analysis. S1P3 mRNA expression in knee joint capsule in CIA mice was about ﬁve times as high as that in normal mice. TNFa treatment upregulated S1P3 expression and S1P treatment enhanced IL-6 production in WT-FLS signiﬁcantly. TNFa-priming enhanced S1P-induced IL-6 production, which is signiﬁcantly higher in WT-FLS than in KO-FLS. This effect was not observed in MCP-1 production of WT-FLS.

Conclusions: S1P3-KO reduced severity of arthritis, inﬂammation and bone erosions in CIA. S1P3 mRNA was upregulated in inﬂamed joint capsule. S1P induces IL-6 production via S1P3 upregulation by TNFa in CIA-FLS. S1P3 inhibition could be a good target of the therapy for arthritis.

REFERENCES:

Disclosure of Interest: None declared

Methods: Synovial fluid mononuclear cells (SFMCs), fibroblast like synovial cells (FLSs) and peripheral blood mononuclear cells (PBMCs) were obtained from a study population consisting of patients with active RA or peripheral SpA with at least one swollen joint (for obtaining synovial fluid) (n=14). SFMCs were cultured for 48 hours with and without addition of a MK2 inhibitor (Celgene) at 1000 nM, 333 nM and 111 nM and supernatants were analysed by the Olink proseek multiplex interferon panel and commercially available ELISA assays. Because FLSs are only found in small amounts among SFMCs, autologous co-cultures of FLS and PBMCs and SFMCs were also used. SFMCs cultured for 21 days were used to study inflammatory macrophage differentiation and osteoclastogenesis.

Results: In SFMCs cultured for 48 hours, the MK2 inhibitor decreased the production of CXCL9 (p<0.001), CXCL10 (p<0.01), HGF (p<0.01), CXCL11 (p<0.01), TWEAK (p<0.05), and IL-12B (p<0.05) and increased the production of CXCL5 (p<0.0001), CXCL1 (p<0.0001), TGFβ (p<0.01), TWEAK (p<0.05), and IL-12B (p<0.05) and increased the production of CXCL9 (p<0.001), CXCL10 (p<0.01), HGF (p<0.01), CXCL11 (p<0.01), LAP TGFβ(p<0.05) dose-dependently after Bonferroni correction (all corrected P values). At the highest concentration, the MK2 inhibitor also decreased MCP-1 production (p<0.05). In FLS-SFMC co-cultures, the MK2 inhibitor decreased MCP-1 production (p<0.05) but did not change the production of DKK1 and MMP3. In FLS-PBMC co-cultures, the MK2 inhibitor decreased the production of MCP-1 (p<0.0001), increased MMP3 production (p<0.05) but did not change DKK1 production. In SFMCs cultured for 21 days as a model of inflammatory macrophage differentiation and osteoclastogenesis, the MK2 inhibitor decreased the production of MCP-1 (p<0.05) and tartrate-resistant acid phosphatase (TRAP) (p<0.05) but did not change the production of IL-10.

Conclusions: This study reveals the effects of a MK2 inhibitor in ex vivo models of immune mediated inflammatory arthritis. The MK2 inhibitor changed the secretory profile of SFMCs and decreased inflammatory osteoclastogenesis. Taken together, this points to a role of this MK2 inhibitor in attenuating inflammatory and destructive arthritis.

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Figure 1. Modified from Wagner & Nebreda, Nature Review Cancer, 2009.