THU0094

ROLE OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 SIGNALLING IN COLLAGEN-INDUCED ARTHRITIS

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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 synovio-cyte is upregulated by TNF α treatment. On the other hand, it is not clarified whether S1P/S1P3 signalling pathway contributes to arthritis in RA.

Objectives: The objective of this study is to investigate the role of S1P/S1P3 signalling 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 wild-type (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 realtime 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 TNF α treatment and measured cytokine produced after S1P treatment with or without TNF α 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. TNF α treatment upregulated S1P3 expression and S1P treatment enhanced IL-6 production in WT-FLS significantly. TNF α -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 TNF α in CIA-FLS. S1P3 inhibition could be a good target of the therapy for arthritis.

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THU0095

DIFFERENTIAL COMPLEMENT ACTIVATION IN RHEUMATOID ARTHRITIS PATIENTS

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Background: An atypical subgroup of patients with seropositive rheumatoid arthritis (RA) has been identified with active disease but normal levels of the acute phase protein C-reactive protein (CRP), considered an accurate marker of disease activity. Previously we identified that patients with normal CRP (nCRP) during flares of RA had an altered immunological profile, had diagnostic delays and seemed to respond less well to conventional treatment compared to RA patients who mounted an appropriately high CRP (hCRP) response. Activation of the complement pathway has been associated with disease activity in RA patients and it is suggested both the presence of autoantibodies and activation of complement are required during the inflammatory processes that lead to joint damage. CRP plays a crucial role in the regulation of complement activation via both the alternative and classical pathways, either through interaction with C4 binding protein or degrading C3 fragment-C3b to C3i via factor H. However, it is not known whether these pathways are altered in RA patients with nCRP compared with hCRP during active flares.

Objectives: To investigate how altered CRP response may differentially regulate C3 cleavage in RA patients with nCRP compared to hCRP levels during flares of RA.

Methods: 24 RA patients with active synovitis were recruited, defined by ≥ 1 joint with Power Doppler detected by US, 15 had normal (n)CRP (≤ 5 mg/L) and 9 had high (h)CRP (> 5 mg/L) levels. Serum and detailed clinical data were collected. 18 age and sex matched healthy donors (HCs) were also analysed. Serum was subjected to SOMAscan Proteomic Assay. Complement components were analysed by Western blot following 1:400 serum dilution and assessed for C3/C3a and albumin expression. Densitometric analysis was applied to the Western blots and the C3a values were normalised against albumin, resultant values were expressed as fold change from HC. Results were correlated with clinical and disease features using linear regression curves in Prism.

Results: Proteomics identified differential expression of complement components in serum from hCRP compared to nCRP patients; specifically a significant upregulation of alternative complement pathway factors (eg Factors I, H and B) was seen in hCRP patients and a downregulation of kallistatin, an inhibitor of the classical pathway in nCRP patients. Average C3 cleavage product was 4.8 (0.18–14.4) for hCRP and 3.05 (0.28–6.6) for nCRP, both significantly higher compared to HCs ($p < 0.01$ HC vs nCRP, $p < 0.05$ HC vs hCRP). The levels of C3 cleavage were then correlated against ESR, CRP and anti- Anti-cyclic citrullinated peptide (CCP) levels in both sets of patients. In hCRP patients, strong correlations ($R^2 > 0.5$) were observed for C3 vs ESR ($p < 0.05$), C3 vs CRP ($p < 0.03$) and ESR vs CRP ($p < 0.0003$) but no correlation was found between C3 and levels of anti-CCP antibodies ($R^2 = 0.208$, $p = 0.255$). In contrast nCRP patients demonstrated a strong correlation between C3 levels vs anti-CCP antibodies ($R^2 = 0.53$, $p < 0.05$) whilst no correlation was seen with CRP or ESR levels ($R^2 = 0.0004$, $p = 0.236$).

Abstract THU0095 – Table 1. A table of proteins related to complement and coagulation found differentially expressed between hCRP and nCRP patients by SOMAscan proteomics.

hCRP	nCRP
Factor H	Kallistatin
Factor B	IL 17F
Factor I	
C3b	
F1xab	
C5a	
C9	
F Xa	
F IX	

Conclusions: Cleavage of complement factor C3 appears to be driven by a different mechanism in hCRP compared to nCRP patients suggesting that complement is activated via different pathways. This supports the hypothesis that nCRP and hCRP patients have an altered disease pathogenesis.

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THU0096

A MAPK ACTIVATED KINASE 2 INHIBITOR ATTENUATES INFLAMMATORY AND DESTRUCTIVE ARTHRITIS IN HUMAN EX VIVO MODELS

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Background: Targeting intracellular pathways with oral small molecules is an attractive therapeutic approach for treating immune mediated inflammatory diseases. The mitogen-activated protein kinase (MAPK) pathway is activated by environmental stressors, growth factors and inflammatory cytokines. However, the inhibition of central MAPK proteins has so far had undesirable side effects. The MAPK-activated protein kinase 2 (MK2) is a downstream mediator in the MAPK signalling pathway and could therefore be inhibited without the same side effects (see figure 1).

Objectives: The objective of this study was to study the effects of a small molecule inhibiting MK2 on inflammation and structural changes in *ex vivo* models of immune mediated inflammatory arthritis.

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 ($p<0.0001$), CXCL6 ($p<0.001$), TGF α ($p=0.01$), MCP-3 ($p<0.01$), LAP TGF β ($p<0.05$) 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.

Abstract THU0096 – Figure 1. Modified from Wagner & Nebreda, *Nature Review Cancer*, 2009.

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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THU0097

PHOSPHODIESTERASES 4 (PDE4) INHIBITOR AMELIORATES EXPERIMENTAL ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory bone-destructive disorder with autoimmune features. Apremilast is a novel phosphodiesterases 4 (PDE4) inhibitor suppressing immune and inflammatory responses.

Objectives: We assessed the anti-inflammatory and bone protection effects of apremilast in collagen CII induced arthritis (CIA) models.

Methods: Apremilast was given starting from day 14 after immunisation, we investigated whether apremilast (5 mg/kg or 25 mg/kg) can ameliorate arthritis onset. Bone erosion was measured by histological and micro-computed tomographic analysis. Anti-mouse type II collagen (CII) antibody levels were measured by enzyme-linked immunosorbent assay. Human cartilage and rheumatoid arthritis (RA) synovial fibroblasts (RASFs) implantation in the severe combined immunodeficiency (SCID) mouse model of RA were used to study the role of apremilast in suppression of RASFs destroying cartilage *in vivo*.

Results: We found that apremilast therapy delayed arthritis onset and reduced arthritis scores in CIA model at a different dose, compared to CIA model and blank vector (figure 1A). Total serum IgG, IgG1, IgG2a, and IgG2b were all decreased in apremilast groups. Furthermore, apremilast can prevent CIA mice from bone erosion by CT analysis. High dose of apremilast (25 mg/kg) was superior to low dose (5 mg/kg) in treating CIA (figure 1B, C). Apremilast treatment can inhibit destroy and migratory ability of RASFs to cartilages. Compared to the model group, Apremilast treatment significantly reduced the invasion scores in both primary implant and contralateral implant.

Conclusions: Our data suggest that apremilast was effective in preventing arthritis and bone erosion in CIA model, implicating a potential promise of therapy on rheumatoid arthritis.

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THU0098

COMBINATION THERAPY OF RAPAMYCIN AND A GLUTAMINE ANTAGONIST FACILITATES THE EXPANSION OF MYELOID-DERIVED SUPPRESSOR CELLS AND AMELIORATES ARTHRITIS IN SKG MICE

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Background: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature cells that increase in the pathological state such as tumour or inflammation and have the immunosuppressive ability. MDSCs have been