OP0268

APOPTOSIS OF SYNOVIAL FIBROBLASTS INDUCED BY P53-DERIVED HYBRID PEPTIDES THROUGH DISSOCIATING THE BINDING OF P73 WITH IASPP TO INCREASE PUMA AND BAX EXPRESSION

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Background: In rheumatoid arthritis (RA) synovial fibroblasts (SFs), mutant p53 can lead to transformation-like features resistant to the apoptosis induction.¹ Deficiency in p53-mediated suppression by its dominant-negative counterpart is observed in human cancers with activating p73 as a therapeutic strategy in such patients.² A p53-derived hybrid peptide 37 amino acid (37AA) can inhibit the p73 binding with inhibitory apoptosis stimulating protein of p53 (IASPP), thus activating the downstream apoptosis signalling pathway in tumour cells.³

Objectives: We hypothesised that p73 is involved in the RA pathogenesis, and examined whether p53-derived hybrid peptides can activate p73 to induce apoptosis of SFs by using adenoviral vectors encoding 37AA (Ad37AA) to transduce SFs *in vitro* and inject collagen-induced arthritis (CIA) joints *in vivo*.

Methods: Mononuclear cells (MNCs) from RA patients before and after receiving the adalimumab therapy were examined for iASPP expression by real-time RT-PCR. Synovial tissues and SFs from RA patients and CIA rats were subjected to immunohistochemical and immunofluorescent staining and real-time RT-PCR for the p73 and iASPP expression. SFs transduced with Ad37AA, were subjected to TUNEL assay for apoptotic status and real-time RT-PCR for the expression of downstream apoptosis signalling molecule PUMA and Bax. SFs were transduced with lentiviral vectors-encoding short hairpin p73 RNA to produce p73-silenced SF transfectants. Therapeutic effects of Ad37AA injection were evaluated on CIA joints. Immunohistochemical staining and TUNEL assay were used to analyse synovial cadherin-11/PUMA/IL-6 expression and apoptotic cells, respectively.

Results: There were reduced iASPP levels by targeting TNF in RA MNCs, and increased p73 with co-localised iASPP expression in synovial lining layers and SFs from RA patients and CIA rats. Enhanced cell apoptosis, increased PUMA and Bax expression and lower iASPP-associated p73 levels were identified in Ad37AA-transduced SFs, and silencing p73 abrogated the increased PUMA and Bax expression. Articular indexes and histologic scores were reduced in Ad37AA-injected joints with decreased SF densities, increased apoptotic cells, higher PUMA expression and lower IL-6 levels.

Conclusions: These results demonstrate that injecting p53-derived hybrid peptides can induce apoptosis of SFs through the activation of p73 in the rheumatoid joint, suggesting that strengthening the p73-dependent apoptotic mechanism is a potential therapeutic strategy in RA patients.

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OP0269

ACTIVATION OF MERTK+CD206+ SUBPOPULATION OF HUMAN SYNOVIAL TISSUE-RESIDENT MACROPHAGES LIMITS INFLAMMATORY RESPONSE

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Background: Current therapies have transformed the management of rheumatoid arthritis. However, there are still a substantial proportion of patients who do not respond to treatments, and among those who respond, only small proportion achieve disease remission. We showed previously that human synovial tissue macrophages (STMs) are a heterogeneous population; and a sub-population of CD206⁺MerTK⁺ positive STMs predominates in RA patients in sustained remission, in contrast to patients with active RA. Their surface receptors, e.g. MerTK, and distinct transcriptome suggest that they may play a pivotal role in re-enforcing joint homeostasis during remission.¹ Thus, we hypothesise that activation of

CD206⁺MerTK⁺ human synovial tissue macrophages contribute to the resolution of inflammation.

Objectives: We aimed to investigate whether activation of MerTK in STM sub-population promotes an anti-inflammatory environment in the synovium.

Methods: Using flow cytometric techniques and with a specific antibody panel design,¹ STMs from digested RA synovial biopsies (n=36) were phenotyped and/or harvested. Patients' group included 28 patients with active disease and 8 patients in remission (DAS28 ESR <2.6 and power doppler negative). After excluding all other cell types, macrophages were gated based on the expression of CD64 and CD11b (CD45^{pos}CD64^{pos}CD11b^{pos}HLA-DR^{pos}).¹ Two distinct sub-populations: CD206⁺MerTK⁺ and CD206⁺MerTK⁻ were sorted and cultured on a collagen coated 96-well plate at 1000 cells per well in the presence of with LPS (10 ng/ml)±Gas6, a MerTK ligand (200 ng/ml), for 24 hour. TNF production was measured with a high sensitivity ELISA.

Results: As previously shown,¹ RA patients in sustained remission have a majority of CD206⁺MerTK⁺ STMs whilst patients with active RA show an increased number of CD206⁺MerTK⁻ macrophages. The percentage of CD206⁺MerTK⁺ macrophages negatively correlated with the disease activity score. Stimulation of FACS-Aria sorted CD206⁺MerTK⁺ and CD206⁺MerTK⁻ macrophages with TLR4 ligand induced TNF production. However, activation of MerTK pathway by Gas6 inhibited LPS-induced TNF production by CD206⁺MerTK⁺ subpopulation.

Conclusions: CD206⁺MERTK⁺ macrophages, which predominate in RA patients in remission, have Gas6-mediated negative feedback mechanism limiting TNF production. Thus, Gas6/MerTK pathway in synovial tissue macrophages could drive the resolution of inflammation and synovial tissue homeostasis. Further functional and transcriptomics studies will reveal the therapeutic potential of CD206⁺MERTK⁺ macrophages.

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OP0270

IGA ANTI-CCP ANTIBODIES ARE DETECTABLE IN THE SALIVA BUT NOT SPUTUM OF INDIVIDUALS AT-RISK OF DEVELOPING RHEUMATOID ARTHRITIS

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Background: Recent evidence suggests the initiation of rheumatoid arthritis (RA) – related autoimmunity may occur by local citrullination at the oral mucosa and lungs. IgA antibodies are the hallmark of mucosal immunity; the majority of saliva IgA antibodies are locally produced whereas IgG antibodies are largely serum derived.¹ Furthermore, IgA anti-CCP antibodies have recently been described in the sputum of at-risk individuals.² The relative importance of the oral and lung mucosa in disease initiation is, however, unclear and the prevalence of saliva and sputum anti-CCP antibodies in the same at-risk individuals has not been reported.

Objectives: To investigate the prevalence of IgA anti-CCP antibodies in the saliva and sputum of seropositive individuals at risk of developing RA.

Methods: Anti-CCP positive individuals with no evidence of clinical synovitis (CCP+), anti-CCP positive RA patients (RA) and healthy controls (HC) matched for age and smoking status were recruited. Unstimulated saliva and serum samples were collected. Induced sputum samples were obtained using 7% saline via ultrasonic nebuliser (UltraNeb 3000 DA, Devilbiss, Germany). Sputum was mixed with phosphate buffered saline, mechanically disrupted and centrifuged to obtain supernatant. IgA and IgG anti-CCP antibodies (anti-CCP2, immunocap assay, Phadia) were measured in all saliva, sputum and serum samples. IgA and saliva/sputum IgG anti-CCP titres exceeding the 95th centile in HC were considered positive.

Results: 55 CCP+, 40 RA and 32 HC were recruited and had saliva and serum collected. 24 CCP+, 14 RA and 22 HC had sputum and serum collected. Of these, 23 CCP + and 7 RA patients provided simultaneous saliva, sputum and serum samples. 8/55 (15%) CCP + and 10/40 (25%) RA patients had positive saliva IgA anti-CCP levels compared with 1/31 (3%) HC. 23/54 (43%) CCP + and 21/48 (44%) RA patients had positive serum IgA anti-CCP levels compared with 1/32 (3%) HC (table 1). Of note, 7/18 (39%) patients with a positive saliva IgA anti-CCP test had a negative serum IgA anti-CCP test, suggesting localised production and accumulation of IgA anti-CCP antibodies rather than transfer from the serum. Only 1/24 CCP+ (4%) and 1/14 (7%) RA patients had positive sputum IgA anti-

CCP levels. No patients had IgA anti-CCP detectable in both saliva and sputum samples.

Abstract OP0270 – Table 1

	Anti-CCP positive results (%)					
	Saliva IgA	Sputum IgA	Serum IgA	Saliva IgG	Sputum IgG	Serum IgG
HC	1/31 ⁽¹⁾	1/22 ⁽¹⁾	1/32 ⁽¹⁾	1/31 ⁽¹⁾	0/22 ⁽⁰⁾	0/32 ⁽⁰⁾
CCP+at-risk	8/55 ⁽¹³⁾	1/24 ⁽¹⁾	23/54 ⁽⁴³⁾	23/54 ⁽⁴³⁾	10/24 ⁽⁴²⁾	50/54 ⁽⁹³⁾
RA	10/40 ⁽²⁵⁾	1/14 ⁽⁷⁾	21/48 ⁽⁴⁴⁾	23/42 ⁽⁵⁵⁾	9/14 ⁽⁶⁴⁾	47/48 ⁽⁹⁸⁾

Conclusions: We found an increased prevalence of saliva but not sputum IgA anti-CCP antibodies in seropositive at-risk individuals. These findings support the concept that localised RA-related autoimmunity in at risk individuals can be site specific. IgA anti-CCP antibodies at the oral mucosa precede arthritis and may represent an important step in the initiation and propagation of disease.

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OP0271

GASTROINTESTINAL DAMAGE AND MICROBIAL TRANSLOCATION ARE INVOLVED IN THE DEVELOPMENT OF IMMUNE SYSTEM ACTIVATION IN INFLAMMATORY BOWEL DISEASE-ASSOCIATED SPONDYLOARTHRITIS

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Background: The altered composition of the gastrointestinal (GI) microbiota, known as dysbiosis, can induce and modulate the systemic inflammation, through microbial translocation and T-cell activation, in several immuno-mediated diseases, such as inflammatory bowel disease (IBD), HIV infection, and ankylosing spondylitis.

Objectives: In a cohort of 85 patients with inflammatory bowel disease-associated spondyloarthritis (SpA/IBD), we assessed gut bacterial infiltration and intestinal damage. In systemic circulation, GI epithelial damage, microbial translocation and immune system activation were assessed with intestinal-fatty acid binding protein (I-FABP), lipopolysaccharide (LPS), soluble CD14 (sCD14), respectively. Moreover, the *in vitro* activity of the latter two was evaluated on osteoblast cells.

Methods: I-FABP, LPS, sCD14, sclerostin (SOST) and anti-SOST antibodies (anti-SOST-IgG) were assayed with ELISAs. LPS and sCD14 were used *in vitro* to stimulate the MG63 human osteoblast-like cell line. Occludin, claudin-1, claudin-4, and the presence of bacteria were assessed, respectively, by real-time-PCR analysis and immunohistochemical staining of the ileum.

Results: Bacteria were detectable in the ileal epithelium of IBD patients, but only in SpA/IBD they were associated with epithelial damage and downregulation of occludin, claudin-1 and claudin-4 (figure 1A-B).

The serum levels of I-FABP, LPS and sCD14 resulted significantly higher in axial (187.9, 14.03, and 26.97, respectively) and peripheral SpA/IBD (130.3, 11.55, and 18, respectively) than in IBD patients (IFABP 43.65, $p<0.0001$ for both patients' groups; LPS 9.625, $p<0.0001$ vs Ax-SpA/IBD and $=0.007$ vs Per-SpA/IBD; sCD14 12.34, $p<0.0001$ for both patients' groups) (figure 1C).

In the SpA/IBD cohort, SOST was weakly correlated with I-FABP ($r=0.2683$), LPS ($r=0.3063$) and sCD14 ($r=0.3075$), and anti-SOST-IgG with LPS ($r=0.3959$) and sCD14 ($r=0.3414$). Moreover, sCD14 showed significant correlation with I-FABP ($r=0.3316$) and LPS ($r=0.5649$).

In vitro, LPS, but not sCD14, significantly induced SOST expression through the upregulation of both Wnt3a and Wnt5a and the downregulation of the b-catenin proteins (figure 1D). On the opposite, the combination of LPS and sCD14 downregulated SOST expression through the upregulation of ERK1/2 and b-catenin protein (figure 1D).

Abstract OP0271 – Figure 1 A. immunohistochemical staining of ileal bacteria. Ten ileal samples of patients affected by SpA/IBD or IBD were stained for bacteria infiltration (upper panel). Lower (from left to right): Gram and LPS staining of the same samples. B. Analysis of ileal tight-junctions proteins expression. From the left to the right: Ten ileal samples of patients affected by SpA/IBD or IBD were stained for occludin (and claudin-1 and -4, data not shown); count of occludin positive cells and quantitative real-time-PCR of occludin (and claudin-1 and -4, data not shown) expression in the same ileal samples. C. analysis of I-FABP, LPS and sCD14 serum levels in SpA/IBD and IBD patients. ELISAs assays were carried out in 45 patients with axial and 40 patients with peripheral SpA/IBD, and compared with IBD or HC. D. Western-blot analysis of MG-63 osteoblast-cells. The MG-63 osteoblast-like cell line was stimulated with LPS $\pm$ sCD14 *in vitro* and then cells were harvested for western blot analysis. Semi-quantitative densitometric analysis of the protein bands was carried out on the blot (data not shown). Statistical analysis: Kruskal-Wallis analysis. ** $p<0.01$; **** $p<0.0001$; if not reported: p not significant. Abbreviations. SpA/IBD: inflammatory bowel disease-associated spondyloarthritis; ax-SpA/IBD: axial SpA/IBD; peripheral SpA/IBD; IBD: inflammatory bowel disease; HC: healthy controls; I-FABP: intestinal fatty acid binding protein; LPS: bacterial lipopolysaccharide; sCD14: soluble CD14; ERK 1/2: extracellular Signal-regulated Kinase-1/2. Wnt: wingless protein family; SOST: sclerostin.

Conclusions: The role of gut inflammation and microbial translocation in the onset of arthritis in IBD patients are still under investigation. We have demonstrated that in SpA/IBD there is a significant bacterial infiltration of the ileal tract, associated with the downregulation of tight-junctions' proteins (occludin, claudin-1 and claudin-4) and epithelial damage, that cause microbial translocation and higher plasma levels of I-FABP, LPS, and sCD14. LPS and sCD14, thus, could trigger a complex systemic inflammatory response acting on several biochemical pathways, linking the immune system (anti-SOST-IgG) and the bone (SOST).

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OP0272

INFLAMMATION AT BARRIER TISSUES SUCH AS SKIN AND GUT TRIGGERS MILD JOINT INFLAMMATION AND IS INFLUENCED BY BIOMECHANICAL STRESS INDUCED BY FORCED-RUNNING

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Background: The factors triggering the onset of psoriatic arthritis (PsA) and other forms of spondyloarthritis (SpA) are mostly unknown. These joint diseases are clinically associated with psoriasis (PsO) and inflammatory bowel disease (IBD). The three pathologies share the common leitmotiv of chronic inflammation and all of them have an at least partially shared genetic susceptibility. Entheses, the attachment sites of tendons and ligaments into the bones, are considered as a primary disease localization and a site of biomechanical stress. Increasing evidence supports the hypothesis that biomechanical stress, together with inflammatory triggers such as antigens and changes in the microbiome, can contribute to the onset of PsA and SpA by inducing local microdamage in the entheses.

Objectives: Here, we aim to understand early events leading to PsA and SpA by combining a protocol of forced exercise in mice with simultaneous locally-induced cutaneous or intestinal inflammation.

Methods: Forty 8 weeks old C57/Bl6 male mice were used to induce the PsO- or IBD-like disease, respectively by serial applications of imiquimod cream (IMQ) on a shaved area of the back skin, and administration to the intestine of dextran sodium sulphate (DSS) dissolved in drinking water. Forty control mice were left