APOPTOSIS OF SYNOVIAL FIBROBLASTS INDUCED BY P53-DERIVED HYBRID PEPTIDES THROUGH DISSOCIATING THE BINDING OF P73 WITH IA5SP TO INCREASE PUMA AND BAX EXPRESSION

C.-R. Wang1, S.-Y. Chen2, A.-L. Shiu3, G.-L. Wu3, 1Internal Medicine, National Cheng Kung University Hospital, 2Microbiology and Immunology, 3Biochemistry and Molecular Biology, National Cheng Kung University Medical College, Taiwan, Taiwan.

Background: In rheumatoid arthritis (RA) synovial fibroblasts (SFs), mutant p53 can lead to transformation-like features resistant to the apoptosis induction. Deficiency in p53-mediated suppression by its dominant-negative counterpart is observed in human cancers with activating p53 (ASPP), thus activating the downstream apoptosis signalling pathway in tumour cells.

Objectives: We hypothesised that p53 is involved in the RA pathogenesis, and examined whether p53-derived hybrid peptides can activate p73 to induce apoptosis of SFs by using adenovector 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 p73SP expression by real-time RT-PCR. Synovial tissues and SFs from RA patients and CIA rats were subjected to immunohistochemical and immunofluorescence staining and real-time RT-PCR for the p73 and IA5SP 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 molecules 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/LIL-6 expression and apoptotic cells, respectively.

Results: There were reduced IA5SP levels by targeting TNF in RA MNCs, and increased p73 with co-localised IA5SP expression in synovial lining layers and SFs from RA patients and CIA rats. Enhanced cell apoptosis, increased PUMA and Bax expression and lower IA5SP-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 decreased IA5SP 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.

REFERENCES:

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.1900

OP2089 IGANGI Anti-CCP Antibodies are Detectable in the Saliva but Not Sputum of Individuals at-Risk of Developing Rheumatoid Arthritis

K. Markevi1, P. Pentony2, L. Hunt3, Y. El-Sherbiny4, L. Duquenne4, D. Consaldien5, T. Do6, J. Meade7, D. Devine7, P. Emery7, 1Rheumatology, Leeds Institute of Rheumat and Musculoskeletal Medicine and NIHR Leeds Biomedical Research Centre, 2Rheumatology, Leeds Institute of Rheumatic and Musculoskeletal Medicine, 3Oral Microbiology, University of Leeds, Leeds, UK

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. Furthermore, IgA anti-CCP antibodies have recently been described in the sputum of at-risk individuals. 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 (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 IgA 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 sputum samples, sputum and serum samples. 8/25 (32%) 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-
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.

REFERENCES:

Disclosure of Interest: None declared


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

D. Bentenatore1, M.M. Luchetti1, F. Ciccia1, C. Avellini1, T. Spadoni1, S. Svegliati1, M. Cifero1, A. Gabrielli1, 1Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche, Ancona; 1Dipartimento Biomedico di Medicina Interna e Specialistica, U.O. di Reumatologa, Palermo, Italy

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 immune-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, inflammation was evaluated in the ileum.

Methods: I-FABP, LPS, sCD14, sclerostin (SOST) and anti-SOST antibodies (anti-SOST-IgG) were assessed with ELISAs. LPS and sCD14 were used in vitro to stimulate the MG-63 human osteoblast-like cell line. Oclucdin, 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 oclucdin, 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 (I-FABP 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=0.2863), LPS (r=0.3063) and sCD14 (r=0.3073), 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 WntSA 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).

Conclusions: The role of gut inflammation and microbial translocation in the onset of arthritis in IBD patients is 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 (occlucdin, claudin-1 and claudin-4) and epithelial damage, that cause microbial translocation and higher plasma levels of I-FABP, LPS, and sCD14. Thus, in SpA/IBD, gut inflammation may trigger a complex systemic inflammatory response acting on several biochemical pathways, linking the immune system (anti-SOST-IgG) and the bone (SOST).

Disclosure of Interest: None declared


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

G.R. Gulino1, M. Van Meehelen2, R. Lories1,2, Skeletology Biotechnology and Engineering, KU Leuven, 2Division of Rheumatology, UZ Leuven, Leuven, Belgium

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 leukotactic 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/B6 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...