root diameter/BSA ratio was significantly higher in HLA-B27 positive patients compared to HLA-B27 negative patients: 1.75±0.22 mm vs. 1.61±0.14 mm (p=0.001). Eight AS patients (6%) had aortic root dilatation (>2.1mm corrected for BSA), who were all HLA-B27 positive. Patients with and without aortic root dilatation did not significantly differ in age or disease duration. The median aortic root diameter/BSA ratio was correlated with disease duration (r=0.229, p=0.012), but not with inflammatory biomarkers.

Conclusions: HLA-B27 positive AS patients with a long disease duration have an increased aortic root diameter and a higher risk for aortic root dilatation compared to HLA-B27 negative AS patients. The risk of developing aortic root dilatation in AS patients is comparable to patients with essential hypertension (6%)[2].

Future prospective studies should assess which AS patients phenotype are at the highest risk of this AS specific cardiac disease, thus candidates for regular echocardiographic screening.

References:

[1] Klingberg et al. Aortic Regurgitation Is Common in AS: Time for Routine Echocardiography Evaluation? Am J Med 2015Nov;128(11)1244–1250.

[2] Cuspidi C et al. Prevalence and correlates of aortic root dilatation in patients with essential hypertension. J Hypertens 2006 Mar;24(3)573–80.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4616

AB0117 CADHERIN-11 MRNA EXPRESSION IS INCREASED IN THE PERIPHERAL BLOOD OF PATIENTS WITH ACTIVE SPONDYLARTHROPATHY: A PILOT STUDY

N.I. Vlachogiannis, P.F. Christopoulos, G. Bamias, K. Fragiadaki, M. Tektonidou, P.P. Sfikakis. *Joint Academic Rheumatology Program, National and Kapodistrian University of Athens Medical School, Athens, Greece*

Background: Cadherin-11 is a key regulator of synovial architecture and has a central role in the formation of the rheumatoid pannus. Immunohistochemical studies have shown upregulation of cadherin-11 in the synovium of patients with seronegative spondylarthropathies (SpA), comparable to that in rheumatoid arthritis (RA), as well as in the intestinal tissue of patients with inflammatory bowel disease (IBD). Moreover, cadherin-11 mRNA transcripts have been identified in the peripheral blood of patients with RA and independently associated, among various disease characteristics, with the presence of active inflammation in multiple joints [1].

Objectives: To test the hypothesis that cadherin-11 mRNA transcripts are increased in the peripheral blood of patients with active SpA and search for possible associations with clinical features.

Methods: Fifteen patients with active SpA (BASDAI>4), aged between 21 and 71 years, 11 men, and 30 age- and gender-matched healthy controls were examined. Peripheral whole blood samples (3 ml) were subjected to cDNA synthesis and cadherin-11 mRNA expression was quantified by real-time PCR. Available cDNA from 33 IBD patients without axial or peripheral active arthritis served as disease control and were studied in parallel.

Results: Cadherin-11 mRNA was detected in the peripheral blood of 9/15 (60%) patients with SpA versus 5/30 (17%) healthy controls and 10/33 (30%) patients with IBD (SpA vs healthy controls p: 0.006, SpA vs IBD p: 0.06). Notably, cadherin-11 was not associated with BASDAI or skin/nail psoriasis present in 10 patients, but was detected with increased frequency among SpA patients with clinically active peripheral arthritis at the time of sampling (7 out of 10, 70%) than the remaining patients (2 out of 5, 40%). Moreover, cadherin-11 positivity associated significantly with increased erythrocyte sedimentation rate in SpA but not in IBD.

Conclusions: Cadherin-11 mRNA is upregulated in the peripheral blood of patients with SpA and may correlate with "spreading" of inflammation in peripheral joints. Since an anti-cadherin-11 mAb is in early clinical development for RA, further studies in patients with inflammatory arthritis are warranted.

References:

[1] Sfikakis PP, Christopoulos PF, Vaiopoulos AG, et al. Cadherin-11 mRNA transcripts are frequently found in rheumatoid arthritis peripheral blood and correlate with established polyarthritis. Clin Immunol 2014;155:33–41. doi:10.1016/j.clim.2014.08.008.

Disclosure of Interest: N. Vlachogiannis: None declared, P. Christopoulos: None declared, G. Bamias: None declared, K. Fragiadaki: None declared, M. Tektonidou: None declared, P. Sfikakis Grant/research support from: Educational grant from Janssen

DOI: 10.1136/annrheumdis-2017-eular.3650

AB0118 THE LYMPHATIC SYSTEM: A GATEKEEPER FOR MIGRATION OF PATHOGENIC T-CELLS TOWARDS SYNOVIAL JOINTS AND ENTHESES IN PSORIASIS

R. Bisoendial[1], E. Prens[2], A. Mus[3], P. Asmawidjaja[3], N. Davelaar[3], A. Hofman[4], J.-B. Jaquet[4], J. Hazes[3], M. Kok[5], E. Lubberts[3]. *[1]Department of Rheumatology, Maasstad hospital and Erasmus Medical Center; [2]Department of Dermatology; [3]Department of Rheumatology, Erasmus University Medical Center; [4]Department of Plastic surgery; [5]Department of Rheumatology, Maasstad hospital, Rotterdam, Netherlands*

Background: The factors underlying the transition of psoriasis (PsO) to psoriatic arthritis (PsA) are poorly understood. The lymphatic system may control the homing of disease-associated T-cells to skin and extra-cutaneous sites like synovial joints and entheses.

Objectives: To study the capacity of lymphatic endothelial cells (LEC) to regulate T-cell homing capabilities in PsA.

Methods: Human dermal LEC ($0.5x10^4$), and fibroblast-like synoviocytes of a patient with PsA (PsA-FLS; $1.0x10^4$) were pre-incubated for 3 days with PsA synovial fluid (PsA-SF; 0/10/20% v/v) and co-cultured with $2.5x10^4$ allogeneic CD4+CD45RO+CD25- T-cells that were sorted from peripheral blood mononuclear cells of 3 donors (with or without stimulation with αCD3/αCD28). After 72 h, T-cells were analyzed by flow cytometry. The CCR6+ T-helper (Th) subsets Th17.1 (CCR4-/CXCR3+), Th17/Th22 (CCR4+/CXCR3-), Th17 (CCR4+/CXCR3-/CCR10-) and Th22 (CCR4+/CXCR3-/CCR10+), and CCR6- subsets Th1 (CCR4-/CXCR3+), and Th2 (CCR4+/CXCR3-) were identified. We also looked at cutaneous lymphocyte- associated antigen (CLA). IL-17A, IL-22, and TNF protein levels were determined by ELISA. Statistical analysis included unpaired t-test (two-sided) for two-group comparison or one-way ANOVA with the Tukey-Kramer post hoc test for multi-group comparisons.

Results: Stimulation of CD4+CD45RO+ T-cells in co-culture with PsA-FLS skewed towards the CCR6+ subset Th17/Th22, which were predominantly Th17 cells. Th17 differentiation upon stimulation was suppressed in co-culture with LEC, even when LEC were pre-incubated with PsA-SF. T-cell stimulation in co-culture with LEC, as compared to PsA-FLS, promoted the generation of the Th22 subset. Upon co-culture with untreated LEC, stimulated T-cells showed higher expression of the skin homing receptor CLA than those that were co-cultured with PsA-FLS. The proportional reduction in CLA expression on T-cells in the co-cultures with LEC pre-incubated with PsA-SF 20% was comparable to PsA-FLS, however LEC conserved CLA expression on CD4+CD45RO T-cells at a higher level than PsA-FLS; this phenomenon particularly affected the CCR6+ T-cells. In line, a trend towards lower IL-17A and higher IL-22 levels were observed in the co-cultures with LEC that were pretreated with PsA-SF 20%, as compared to the co-culture with PsA-FLS. No differences were seen for TNF protein levels.

Conclusions: LECs are directly involved in T-cell differentiation and homing capabilities, as shown by suppression of Th17 differentiation upon stimulation, as compared to PsA-FLS. Also, LEC promoted Th22 generation, and conserved CLA expression in CCR6+ T-cells, even when LEC were preincubated with PsA-SF. Studies are underway to confirm that LECs from relevant biological tissues (e.g. synovium and lymph nodes) are critical for tissue-restricted T-cell migration to skin and synovial membranes in PsA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5360

AB0119 ANTI-FIBROTICS TO PREVENT NEW BONE FORMATION IN SPONDYLOARTHRITIS: PROOF OF CONCEPT USING PIRFENIDONE IN CELL CULTURE MODELS

J. Laustsen[1,2], S. Lomholt[3], P. Andersen[3], J. Kelsen[4], T.W. Kragstrup[1]. *[1]Department of Rheumatology, Aarhus University Hospital; [2]Department of Biomedicine; [3]Aarhus University; [4]Aarhus University Hospital, Aarhus C, Denmark*

Background: The pathogenesis of spondyloarthritis (SpA) involves both inflammation and new bone formation in the spine. In line with this, the disease has been characterized as both inflammatory and fibrotic. Current treatment including inhibitors of tumour necrosis factor alpha (TNFα) seem to dampen inflammation while new bone formation can progress. Therefore, there is an unmet therapeutic need for the treatment of new bone formation in SpA. Fibrosis is mediated by myofibroblasts and new bone formation is the result of increased osteoblast mineralization and decreased osteoclast bone degradation. Here, we evaluate the potential effect of the newly approved anti-fibrotic agent pirfenidone (Esbriet, Pirespa) on fibrosis and new bone formation in cell culture models of SpA.

Objectives: We hypothesized that pirfenidone inhibits SpA myofibroblast formation and activity and osteoblast mineralization.

Methods: Synovial fluid mononuclear cells from patients with SpA (n=6) were included for culturing fibroblast-like synovial cells (FLSs) while osteoblasts were purchased. The cells were cultured with pirfenidone in increasing concentrations (0.25, 0.5, and 1.0 mg/ml) with or without stimulation with tumor necrosis factor alpha (TNFα), transforming growth factor beta (TGFβ), or interferon gamma (INFγ). The proliferation of FLSs was analyzed with light microscopy and flow cytometry using the marker Ki67. The differentiation and activation of FLSs was assessed with flow cytometry, a proteome profiler assay and enzyme-linked immunosorbant assays. The mineralization capacity of the osteoblasts was measured as deposition of hydroxyapatite.

Results: Pirfenidone reduced the Ki67 expression 7.1-fold in untreated FLSs (p=0.001) and 11.0-fold in FLSs stimulated with TGFβ, TNFα, and INFγ (p=0.022). PFD further inhibited TGFβ induced upregulation of αSMA (Figure 2A) and INFγ induced upregulation of HLA-DR (Figure 2C) in all cultures. There was no difference between the percentage of ICAM-1 positive FLSs in cultures treated with or without pirfenidone. In supernatants from FLSs stimulated with TGFβ, TNFα, and INFγ a total of 12 cytokines or chemokines had values above the detection limit in the membrane-based antibody array. Pirfenidone decreased the secretion of 3 of these 12 cytokines or chemokines more than 2-fold. The changes in secretion of monocyte chemoattractant protein 1 (MCP-1) and chitinase-3-like

protein 1 (CHI3L1, also known as YKL-40) were validated with ELISA. Further, pirfenidone decreased the secretion of both DKK1 (p=0.006) and OPG (p=0.02) by SpA FLSs stimulated with TGFβ, TNFα, and INFγ, while the concentration of RANKL was below the detection limit of the ELISA assay in all cultures. Finally, pirfenidone inhibited the deposition of hydroxyapatite by osteoblasts in a dose-dependent manner (p=0.0001). This inhibition was partly reversible when removing pirfenidone after the first week of the mineralization assay.
Conclusions: Taken together, pirfenidone inhibited SpA myofibroblast formation and activity and osteoblast mineralization. This encourages further research in anti-fibrotics as treatment of new bone formation in SpA.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1503

AB0120 ACCELERATED OSTEOGENIC DIFFERENTIATION OF HUMAN BONE-DERIVED CELLS IN ANKYLOSING SPONDYLITIS

Y.-S. Park[1], S. Jo[2], I.-H. Sung[3], T.-H. Kim[2]. *[1]Orthopaedic Surgery, Hanyang University Hospital, Guri; [2]Rheumatology, Hanyang University Hospital for Rheumatic Disease; [3]Orthopaedic Surgery, Hanyang University Hospital, Seoul, Korea, Republic Of*

Background: Ankylosing spondylitis (AS) is characterized by excessive bone formation with syndesmophytes, leading to bony ankylosis. The contribution of osteoblasts to the pathogenesis of ankylosis is poorly understood.
Objectives: The aim of this study was to determine molecular differences between disease controls (Ct) and AS bone-derived cells (BdCs) during osteogenic differentiation.
Methods: We confirmed osteoblastic differentiation of Ct and AS BdCs under osteogenic medium by observing morphological changes and measuring osteoblastic differentiation markers. Osteoblast differentiation was detected by alkaline phosphatase (ALP) staining and activity, and alizarin red S and hydroxyapatite staining. Osteoblast-specific markers were analyzed by qRT-PCR, immunoblotting, and immunostaining. To examine the effects of inflammation, we added AS and healthy control serum to Ct and AS BdCs, and then analyzed osteoblast-specific markers.
Results: AS BdCs showed elevated basal intercellular and extracellular ALP activity compared to Ct. When osteoblast differentiation was induced, AS BdCs exhibited higher expression of osteoblast-specific marker genes and faster mineralization than Ct BdCs, indicating that these cells differentiated more rapidly into osteoblasts. ALP activity and mineralization accelerated when serum from AS patients was added to Ct and AS BdCs.
Conclusions: Our results revealed that AS BdCs showed significantly increased osteoblastic activity and differentiation capacity by regulating osteoblast-specific transcription factors and proteins compared to Ct BdCs. Active inflammation caused by adding AS serum accelerated bony ankylosis. Our study could provide useful basic data for understanding the molecular mechanism of ankyloses in AS.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4885

SLE, Sjögren's and APS - etiology, pathogenesis and animal models

AB0121 DYSREGULATED CIRCULATING MIRNA LEVELS ARE CHARACTERISTIC OF BOTH NON SJÖGREN'S SICCA AND PRIMARY SJÖGREN'S SYNDROME PATIENTS

A.P. Lopes[1,2], M.R. Hillen[1,2], E. Chouri[1,2], S.L. Blokland[1,2], A.A. Kruize[1], M. Rossato[1,2], T.R. Radstake[1,2], J.A. van Roon[1,2]. *[1]Department of Rheumatology & Clinical Immunology; [2]Laboratory of Translational Immunology, University Medical Centre Utrecht, Utrecht, Netherlands*

Background: MicroRNAs are small non-coding RNAs that play important regulatory roles in a variety of biological processes. They can regulate the post–transcriptional expression of target genes and play an important role in gene regulation. Specific microRNAs are stably present in serum and changes in their abundance are potentially disease-specific. Considering their important role in regulation of the immune system, we investigated circulating levels of miRNAs in patients with primary Sjögren's syndrome (pSS) and those with non-Sjögren's sicca (nSS) in relation to disease activity.
Objectives: To assess the expression of a large number of miRNAs in the serum of pSS and nSS patients as compared to healthy controls and to investigate their correlation with disease activity.
Methods: Two independent cohorts (discovery and validation) were established, consisting of a total of 37 pSS patients classified according to the 2002 criteria, 20 nSS patients that were not clinically considered to be pSS and did not meet the classification criteria, and 18 healthy controls (HC). Serum miRNAs were isolated and miRNA profiling of 758 miRNA was performed using the OpenArray platform in the discovery cohort. A selection of 10 miRNAs found to be differentially expressed between the groups was measured in the independent validation cohort using single TaqMan microRNA Assays.
Results: miRNA profiling revealed 10 miRNAs to be differentially expressed between the groups; 2 in pSS vs HC, 7 in nSS vs HC and 1 in both pSS and nSS vs HC. One miRNA was excluded from further analysis after technical validation by single TaqMan microRNA Assay. The other 9 miRNAs were measured in the validation cohort. Surprisingly, 2 miRNAs were validated to be increased in the nSS group as compared to HC (snRNA-U6 and miR-661). Using the data from both cohorts combined, the levels of snRNA-U6 and miR-661 was associated with serum Ig and C4 in the nSS group, but also in the pSS group. This prompted us to investigate miRNA expression in subgroups of pSS patients. snRNA-U6 and miR-661 levels are significantly increased compared to HC in pSS patients negative for autoantibodies. In autoantibody positive pSS patients, levels of snRNA-U6 and miR-661 are comparable to those found in HC and both miRNAs are significantly increased in autoantibody negative patients as compared to autoantibody positive pSS patients. In addition, their expression is strongly associated with leukocyte numbers in the autoantibody positive patients, but not in the negative patients.
Conclusions: Increased circulating levels of snRNA-U6 and miR-661 in patients with nSS and autoantibody negative pSS patients are associated with normal B cell activity and normal numbers of circulating leukocytes. Reduced miRNA levels in autoantibody positive pSS patients are associated with B cell hyperactivity and decreased leukocyte counts, which is possibly the result of immune cells migration to the inflammatory sites. Considering the important role of miRNAs in the control of immune cell activation, this work points to a significant role of miRNAs in pSS and nSS patients.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4961

AB0122 DETECTION OF AN INTRIGUING VIRUS-LIKE SEQUENCE IN THE SALIVARY GLAND EPITHELIAL CELLS OF SJÖGREN'S SYNDROME PATIENTS

A.G. Vakrakou[1,2], E. Karamichali[3], O. Georgopoulou[3], M.N. Manoussakis[1,2]. *[1]Pathophysiology, University of Athens; [2]Laboratory of molecular immunology; [3]Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece*

Background: Several previous studies from our laboratory had indicated that the salivary gland epithelial cells (SGEC) of primary Sjögren's syndrome (SS) patients manifest cell autonomous (intrinsic) activation, which is demonstrated in non-neoplastic cell lines derived from patients (SS-SGEC). As for other autoimmune diseases, it is widely proposed that infectious factors may incite SS. In fact, several viral agents have been proposed to be involved in the development of SS, including EBV, retroviruses (such as HTLV-1 and endogenous retrotransposons) and the Coxsackie virus. Recently, the sequence and antigen of HDV were detected in the salivary gland tissues of approximately half of SS patients studied [Weller et al., 2016], however, in the absence of detectable hepatitis B virus (HBV) surface antigen and antibodies to HBV or HDV. Therefore, we hypothesized that HDV may be causally related with the cell-autonomous aberrations observed in the SS-SGEC lines and may trigger the inflammatory and autoimmune reactions that characterize SS.
Objectives: Herein, using non-neoplastic SGEC lines derived from SS patients, we sought to address directly whether the SGEC of these patients manifest evidence for infection by the HDV virus, despite the fact that patients studied did not display any evidence of infection by HBV or HDV viruses.
Methods: For that purpose, we purified total RNA from SGEC lines from non-SS controls (n=7) and SS patients (n=7). Reverse transcription of RNA isolated from salivary gland epithelial cells (SGEC) was performed using random hexamer primers as per the manufacturer's specifications. We performed PCR for the identification of HDV RNA sequences with three sets of primers from different regions of the viral sequence. RNA extracted from HDV-positive sera was used as positive control in HDV-specific PCR.
Results: All SGEC lines from SS patients and non-SS controls tested were found positive for HDV RNA, as tested by the first two sets of primers (two sets of primers for first and nested PCR with expected PCR products 329bp and 234bp, respectively). Nevertheless, when we selected primers for another region of the HDV genome (set of primers with expected PCR products 375bp) all RNA samples from SGEC lines tested were found negative for HDV RNA, whereas the HDV RNA control specimen was positive.
Conclusions: We conclude that SS-SGEC are not actually infected by the HDV virus, but are probably carriers of an HDV-like sequence. In fact, the putative HDV virus-like sequence has been previously shown be highly homologous to the RNA binding-protein cytoplasmic polyadenylation element-binding protein-3 (CPEB3), which belongs to a family of genes regulating messenger RNA polyadenylation and is structurally and biochemically related to the human HDV ribozymes (Salehi-Ashtiani et al, 2006). We are currently in the process of further analyzing the nature of this HDV-like sequence, through cloning and Sanger sequencing of the RT-PCR products, as well as performing of HDV-specific immunostaining studies in both SGEC lines and salivary gland tissues.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6308