Abstract OP0153 – Table 1

	Overall	Patients with infection	Respiratory	Gastrointestinal	Genitourinary	Musculoskeletal	Sepsis	Skin/Soft Tissue	Other
Age, years (SD)	57.3 (12.5)	61.5 (11.7)	60.0 (11.5)	61.7 (11.1)	59.8 (11.9)	58.7 (10.5)	63.5 (11.2)	57.9 (11.9)	55.3 (11.4)
Female (%)	75.7	72.6	74.8	77.4	89.2	70.2	76.6	79.5	83.3
Baseline DAS28 (SD)	6.2 (1.2)	6.4 (1.2)	6.3 (1.2)	6.3 (1.1)	6.3 (1.2)	6.5 (1.2)	6.4 (1.1)	6.4 (1.1)	6.3 (1.1)
Baseline HAQ (SD)	1.9 (0.7)	2.1 (0.6)	2.0 (0.6)	2.1 (0.6)	2.1 (0.6)	2.1 (0.6)	2.2 (0.5)	2.0 (0.6)	1.9 (0.6)
Steroid use, n (%)	8346 (38.2)	2632 (49.4)	2832 (43.8)	493 (51.5)	1226 (44.5)	390 (51.6)	316 (57.3)	1583 (45.2)	1267 (39.6)
Current smoker, n (%)	4682 (21.7)	1159 (21.9)	1422 (22.1)	165 (17.3)	452 (16.4)	186 (24.8)	122 (22.4)	692 (19.9)	573 (18.0)
RhFactor positive, n (%)	13,340 (63.2)	3533 (67.5)	4176 (66.1)	626 (66.2)	1737 (64.6)	527 (71.4)	361 (66.1)	2241 (65.6)	2003 (64.0)
Recurrent infection rate, % per annum (95% CI)	—	12.7 (12.1–13.2)	15.0 (13.9–16.2)	10.8 (9.2–12.8)	14.5 (12.7–16.5)	10.1 (8.7–11.8)	19.7 (15.1–25.7)	11.2 (10.1–12.5)	9.1 (7.3–11.4)
Adjusted Hazard Ratio for annual incidence of recurrent infection (95% CI)	—	—	REF	0.79 (0.65–0.95)	0.97 (0.83–1.14)	0.81 (0.67–0.96)	1.33 (1.01–1.76)	0.87 (0.77–1.0)	0.70 (0.54–0.91)

index infection, the annual rate of serious infection was 12.7% (95% CI 12.1–13.3). Respiratory infections were the most common (41.4% of all events). The system class of index infection was associated with the risk of a recurrent event; subjects who experienced sepsis had the highest risk of subsequent serious infection within 12 months:19.7%. Compared to an index respiratory tract infection, sepsis conferred a 33% increased hazard for recurrent serious infection within a year (HR 1.33, 95% CI 1.01–1.76). Increasing age was a significant predictor of infection recurrence.

Conclusions: There is a high risk of recurrent infection in RA patients with past serious infection. Work is ongoing to determine whether organ class of recurrent infection event mirrors index events and the impact of biologic treatment decisions following the index infection.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1501

THURSDAY, 15 JUNE 2017

Advances in RA and SpA pathophysiology

OP0154 ALTERED LYMPH NODE STROMAL CELLS DURING THE EARLIEST PHASES OF RHEUMATOID ARTHRITIS

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Background: Lymph node stromal cells (LNSC) play a crucial role in shaping the immune response and maintaining peripheral tolerance. We developed an experimental model for studying the functional capacities of human LNSC during the earliest phases of RA and compared their cellular and molecular characteristics to LNSC from healthy volunteers.

Methods: ACPA+ RA patients (n=24), ACPA+ RA-risk individuals (n=23) and seronegative healthy controls (n=14;HC) underwent ultrasound-guided inguinal lymph node biopsy. Human LNSCs were isolated and expanded *in vitro* for cellular (flow cytometry), molecular (methylome, transcriptome and microRNA) and functional (contraction) analyses.

Results: RNA sequencing was performed on LNSC of HC (n=5), ACPA+ RA-risk individuals (n=6) and ACPA+ RA patients (n=4). Of interest, LNSC from ACPA+ RA-risk individuals and ACPA+ RA patients were more similar to each other compared with HC. Pathway analysis of commonly increased genes in RA (-risk) LNSC showed, among others, significant enrichment of pathways affecting actin cytoskeleton, focal adhesion and cell junction.

DNA methylation (Illumina HumanMethylation450 array) analyses revealed 459 differentially methylated CpG sites (DMS) in LNSC from ACPA+ RA patients (n=5) versus HC (n=4), 504 DMS between ACPA+ RA-risk individuals (n=3) versus HC and 665 DMS when comparing RA patients with RA-risk individuals (delta β -value >0.1, p<0.05). 34 DMS were different in both RA and RA-risk LNSC compared to healthy LNSC. 80% of these DMS were significantly hypomethylated and associated with antigen processing and presentation (HLA-DRB1), immune response and regulation of actin cytoskeleton.

Accordingly, in a gel contraction assay LNSC from ACPA+ RA-risk individuals and RA patients showed impaired collagen contraction compared to healthy LNSC. Healthy LNSC (n=5) covered 26.5% $\pm$ 2.5 of the well, while RA-risk (n=4) and RA (n=5) LNSC only covered 33.9% $\pm$ 5.9 and 30.6% $\pm$ 6.5.

Conclusions: This data point towards alterations in the cytoskeleton and antigen-processing and presentation in LNSC from ACPA+ RA-risk individuals and RA patients. Further studies are required to investigate the influence of this LNSC abnormality on immune responses.

Disclosure of Interest: C. Ospelt: None declared, E. Karouzakis: None declared, J. Hähnlein: None declared, J. Semmlink: None declared, R. Gay: None declared, P. Tak Employee of: GSK, D. Gerlag Employee of: GSK, S. Gay: None declared, L. van Baarsen: None declared

DOI: 10.1136/annrheumdis-2017-eular.6208

OP0155 MDM2-MEDIATED SUMOYLATION OF P53 CONTRIBUTES TO THE MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Fibroblast-like synoviocytes (FLSs) in the synovial intimal lining are essential in the maintenance of synovial inflammation and progressive joint destruction in the development of Rheumatoid arthritis (RA). However, the precise molecular mechanisms by which this pathogenic process is regulated are not clearly defined.

Objectives: Increasing evidence indicates that p53 plays a critical role in the invasion and metastasis of cancer cells. This study aims to investigate the role of MDM2-mediated sumoylation of p53 in regulating the migration and invasion of fibroblast-like synoviocytes (FLSs) from patients with RA.

Methods: Synovial tissues were obtained from OA and RA patients, and then FLSs were separated from synovial tissues. Protein expression was measured by Western blotting or IHC staining. The sumoylation of p53 in cells was determined by immunoprecipitation. A specific inhibitor of MDM2-p53 interaction was used to inhibit the sumoylation of p53. Migration and invasion of FLSs *in vitro* were measured by the Boyden chamber assay.

Results: MDM2, SUMO1, and SUMO2 expression was significantly increased in the synovial tissue and FLSs of RA patients. Stimulation with TNF- α increased MDM2 expression and p53 sumoylation in RA FLSs. MDM2 shRNA inhibited p53 sumoylation, pro-inflammatory cytokines and MMPs expression, and capacity of *in vitro* migration and invasion in RA FLSs. Inhibition of p53 sumoylation by MDM2 shRNA promoted apoptosis and reduced proliferation of RA FLSs. p38 MAPK signal pathway was involved in the downstream signal transduction in RA FLSs. Administration of MDM2 shRNA expressing lentivirus attenuate the severity of rats with collagen-induced arthritis (CIA).

Conclusions: Increased MDM2-mediated p53 sumoylation contributes to aberrant aggressive behaviours of RA FLSs. Our findings suggests inhibition of MDM2 or p53 sumoylation might be a novel therapeutic strategy to prevent synovial invasiveness and joint destruction in RA.

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Acknowledgements: This work is supported by grants from the National Natural Science Foundation of China (Grant number 81373182, 81373194, 8150060222 and U1401222). We would like to thank Jinjin Fan for her technical assistance.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.1554

OP0156 METHOTREXATE INCREASES EXPRESSION OF THE CELL CYCLE REGULATORS LBH AND P21 AND REDUCES FIBROBLAST-LIKE SYNOVIOCYTE PROLIFERATION AFTER MITOGEN STIMULATION

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Background: Activated fibroblast-like synoviocytes (FLS) are key effector cells in the joint in rheumatoid arthritis (RA). Local FLS proliferation is responsible for synovial hyperplasia, a key feature of the RA synovium correlating with disease activity. PDGF and IL-1b are known FLS mitogens. LBH is a transcription regulator and tumor suppressor, recently identified as a RA risk gene. We have demonstrated that LBH regulates FLS proliferation and that LBH expression is regulated by growth factors and by epigenetic mechanisms[1]. Methotrexate (MTX) is still the first-line treatment of RA but the target cells and mechanism of action of the low dose used in rheumatic diseases is largely unknown. Increased expression of cell cycle checkpoint genes[2] and modified DNA methylation[3] in immune cells have recently been described.

Objectives: The aim of this study was to investigate the effects of MTX on PDGF and IL1b-induced FLS proliferation *in vitro* and in particular on the expression of LBH, cell cycle genes (CDKN1A/p21 and CCND1/cyclinD1) and on genes regulating DNA methylation (DNMTs) in order to further understand the pharmacodynamics of this drug in RA and to identify novel markers for drug response.

Methods: Primary FLS from RA patients and from patients with osteoarthritis (OA) were plated on day 0 in DMEM complete, pre-treated 24 hours with MTX or control medium day 1, and stimulated with 20ng/ml PDGF+2 ng/ml IL-1b with or without 1 uM MTX in DMEM with 1% FBS for 24–48 hours starting day 2. Cells were then harvested for qPCR for gene expression and flowcytometry for cell cycle analysis.

Results: Stimulating RA-FLS cultures (n=3) with PDGF+IL-1b for 24 hours, pushed 24,5±3,5% cells into G2/M phase compared to 3,4±0,8% in unstimulated controls. Interestingly, treating PDGF+IL1b stimulated FLS with MTX, significantly inhibited cell cycle progression (4,6±1,9% in G2/M phase, p=0,02). PDGF+IL-1b stimulation of FLS for 24 hours reduced LBH mRNA expression. However, in the presence of 1uM MTX the LBH mRNA expression was significantly higher in RA-FLS (3,2±0,5 fold, p=0.002, n=5) and in OA-FLS (2,2±0,5 fold, p=0,02, n=5) after PDGF+IL-1b stimulation compared to untreated controls. In addition, MTX treatment strikingly increased the CDKN1A expression 14,3±4,4 fold (p=0,006) of treated vs untreated stimulated FLS. Furthermore, we found that 1 uM MTX restored and increased a lowered DNMT1 mRNA expression to 144±12% after PDGF+IL1b stimulation. There were no significant effects of MTX on CCND1 or DNMT3a expression at investigated time points.

Conclusions: Therapeutic doses of MTX reduce mitogen induced FLS proliferation and significantly revert mitogen-induced reduction of LBH and p21 expression in RA FLS. MTX restores expression of DNMT1 suggesting that MTX might regulate gene expression and proliferation by affecting the epigenome.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5079

OP0157 APOPTOSIS RESISTANCE OF SYNOVIAL FIBROBLASTS OF PATIENTS WITH RHEUMATOID ARTHRITIS IS REGULATED BY THE LONG NON-CODING RNA FAS-AS1

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Background: Apoptosis resistance is thought to contribute to the accumulation of synoviocytes in the affected joints of patients with rheumatoid arthritis (RA). Of particular interest, the Fas receptor (FasR) - Fas ligand (FasL) apoptotic pathway appears altered in RA¹. Long non-coding RNAs (lncRNAs) are emerging as key regulators of gene expression. Their role in disease, however, is still poorly understood. The recently described lncRNA FAS-AS1 has been implicated in alternative splicing of FasR. This results in increased amounts of soluble FasR (sFasR) and thereby prevents FasL-induced cell death^{2,3}. Whether lncRNA FAS-AS1 is involved in the apoptosis resistance of synovial fibroblasts in RA is unknown.

Objectives: To assess the regulatory role of lncRNA FAS-AS1 in the apoptosis resistance of synovial fibroblasts from patients with RA (RASf).

Methods: Levels of expression of lncRNA FAS-AS1 were measured in RASf and synovial fibroblasts from patients with osteoarthritis (OASf) by qPCR using SYBRGreen detection. Cells were treated with TNFα (10ng/ml, 24h) and/or with FasL (50ng/ml, 18h) to assess the secreted amount of sFasR by ELISA and the induction of apoptosis by Annexin V staining followed by flow cytometry. lncRNA FAS-AS1 was silenced using locked nucleic acid antisense oligonucleotides (GapmeR).

Results: There was no significant difference in basal levels of lncRNA FAS-AS1 expression between RASf and OASf (n=4 each). TNFα stimulation of synovial fibroblasts, regardless of the disease context (RA or OA), resulted in higher than 6-fold induction of lncRNA FAS-AS1 expression (6.45±1.39; p<0.05; n=4 for RASf and 6.26±1.47; p=0.05; n=4 for OASf). In addition, TNFα stimulation induced secretion of sFasR in RASf from 107±74 to 390±274pg/ml (p<0.05; n=6) and OASf from 69±54 to 249±134pg/ml (p<0.05; n=6). FasL induced apoptosis in both RASf and OASf (55–75±13%). However, pretreatment with TNFα reduced the FasL-induced apoptosis in RASf by 25±19% and in OASf by 15±10%. Silencing with GapmeR successfully decreased the expression of lncRNA FAS-AS1 by 40±22% SEM. Most interestingly, silencing of lncRNA FAS-AS1 reverted the TNFα inhibitory effect on FasL-induced apoptosis by 37±11% (n=4).

Conclusions: Our data revealed a novel mechanism, which may underlie apoptosis resistance in RASf. We showed that in a pro-inflammatory cytokine milieu, lncRNA FAS-AS1 up-regulates the release of sFasR and thereby may lower the responsiveness of cells to death signals. Thus, targeting lncRNA FAS-AS1 might prevent apoptosis resistance and synovial hyperplasia in RA.

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Disclosure of Interest: A. Pajak: None declared, Y. Horai: None declared, E. Pachera: None declared, M. Brock: None declared, S. Gay: Grant/research support from: EU project BTCure, IAR, Consultant for: GSK, M. Neidhart: Grant/research support from: Baugartenstiftung, O. Distler: Grant/research support from: Actelion, Bayer, Boehringer Ingelheim, Pfizer, Sanofi, Consultant for: 4 D Science, Actelion, Active Biotech, Bayer, BiogenIdec, BMS, Boehringer Ingelheim, ChemomAb, EpiPharm, espeRare foundation, Genentech/Roche, GSK, Inventiva, Lilly, medac, Mepha, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Pfizer, Sanofi, Serodapharm, Sinox, Speakers bureau: AbbVie, iQone Healthcare, Mepha, A. Jungel: None declared

DOI: 10.1136/annrheumdis-2017-eular.2677

OP0158 PREVALENCE OF IMMUNIZATION OF PATIENTS WITH AUTOIMMUNE DISEASE IN MEXICO

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Background: Current guidelines recommend immunization in patients with autoimmune diseases and use of immunosuppressants including biological treatment. Despite the above, the frequency of immunization is unknown in our population.

Objectives: To identify the prevalence of immunization in patients with autoimmune disease in a Rheumatology Service of a third level hospital in Mexico.

Methods: Observational, descriptive, cross-sectional study. Consecutive outpatients with autoimmune diseases who attended the Rheumatology Service of the Hospital Civil of Guadalajara during a period of 2 months (Dec. and Jan.) were included. A questionnaire was carried out to obtain demographic and immunization data. Descriptive statistical analysis was performed.

Results: 1208 patients were surveyed, 484 (40%) had a diagnosis of autoimmune disease; of whom 286 (59%) were on immunosuppressant therapy. 321 patients had a complete immunization during childhood. None of the patients knew what vaccines should be received with their diagnosis. When asked if they had been invited for immunization and from whom, 24 reported that their