healthy control subjects and DNA microarrays were performed. Flow cytometry and real time PCR were used to verify the expression of de-regulated genes in RA pDCs. Finally, *in vitro* cultures of pDCs activated with CpG A in the presence or absence of recombinant IL-6 (rIL-6) were performed to assess the functional importance of these gene signatures.

Results pDCs from RA patients (n=5) exhibited a differential gene signature (6741 deregulated genes) compared to pDCs from healthy controls (n=5). Notably, IL-6 receptor (IL-6R) gene, exhibited increased expression levels in pDCs isolated from RA patients compared to healthy pDCs and the surface expression levels of IL-6 receptor were verified in a subsequent cohort of patients responding to therapy (n=9) versus active patients or healthy donors. Moreover, assessment of IL-6 signalling pathway in RA patients versus healthy donors revealed a significant increase of pSTAT1 expression levels in RA patients (n=9) compared with healthy donors (n=6) (MFI±SEM, 7.98 ±0.8 versus 12.65±1.18, p=0.0076). Importantly, IL-6-treated pDCs exhibited a vast decrease in TNF-α production (p=0.0002) whereas no differences were found in the production of IFN-α and in their antigen presenting capacity between CpG-treated pDCs in the presence or absence of rIL-6. Moreover, confocal experiments in progress will assess the expression levels of TNF-α in pDCs isolated from RA patients in remission versus active or healthy donors. The functional importance of the previous findings will be addressed in coculture experiments of IL-6 stimulated pDCs with neutrophils isolated from healthy donors and monitor the neutrophil extracellular trap formation.

Conclusions We found that pDCs from RA patients in remission display increased IL-6R expression levels and an activated IL-6 signalling pathway. Activation of IL-6 signalling on pDCs *in vitro* significantly decreases the production of TNF-α whereas it does not alter IFN-α production and their antigen presenting capacity. This novel finding that may drive pDCs towards a previously described tolerogenic phenotype, need to be further addressed.

Disclosure of interest None declared

P010 ABSTRACT WITHDRAWN

P011 EXPANDED T-CELL CLONES ARE PRESENT IN THE SYNOVIUM BEFORE THE ONSET OF CLINICAL RHEUMATOID ARTHRITIS

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Introduction T-cells are thought to be key players in the initiation and progression of rheumatoid arthritis (RA). Earlier we showed that already at the seropositive *at-risk* stage uninflamed synovial tissue contains T-cell infiltrates.[1] In another study we showed that inflamed synovium harbours expanded T-cell clones.[2]

Objectives Following up on these observations, we longitudinally investigated whether the expanded T-cell clones found in the inflamed synovial tissue at onset of RA are already present in the *at-risk* stage.

Methods Next-Generation Sequencing of the TCRβ repertoire was performed on 20 randomly selected individuals with elevated IgM-RF and/or ACPA levels. Ten individuals did not develop RA during at least 3 years of follow-up, and 10 individuals did. Peripheral blood and synovial tissue samples were analysed during the *at-risk* phase and, for individuals that developed RA, again after RA onset. T-cell clones were identified by their unique TCRβ sequence.[3] For each sample 3,570 TCRβ sequences were analysed.

Results During the *at-risk* phase the TCRβ repertoire in the synovium is characterised by expanded clones. This is observed both in *at-risk* individuals that did and did not develop RA. Interestingly, a higher impact of expanded clones inversely correlated with a longer disease-free follow-up time (p=0.02). During progression to RA, the *at-risk* TCRβ repertoire is largely maintained in the tissue. Further characterisation of the synovial CDR3 sequences showed no significant differences between clones that were maintained in the tissue during progression to clinical disease and clones that were uniquely present in the *at-risk* phase or at RA onset.

Conclusions Expanded T-cell clones are present in the synovial tissue in the *at-risk* phase regardless of future development of RA, and are maintained after onset of clinical disease. The resemblance in TCRβ repertoires indicates that the process leading to disease – at least at the T-cell level – constitutes a smooth development. Elucidating the role of these synovial T cells (resident memory, regulatory or autoreactive) might help understanding the earliest pathogenic events in RA.

REFERENCES

1. de Hair MJ, *et al*. *Arthritis Rheum* 2014.
2. Klarenbeek PL, *et al*. *Ann Rheum Dis* 2012.
3. Klarenbeek PL, *et al*. *Immunol Lett* 2010.

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Disclosure of interest None declared

P012 PEPTIDYL ARGININE DEIMINASE IMMUNISATIONINDUCES ANTI-CITRULLINATED PROTEIN ANTIBODIES IN HLA-DRB1*04:01 TRANSGENIC MICE

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Introduction Autoantibodies to citrullinated proteins (ACPAs) are highly associated with rheumatoid arthritis (RA). ACPAs are produced in the absence of T cell responses to citrullinated proteins.

Objectives Peptidyl arginine deiminase 4 (PAD4), which binds numerous different proteins and citrullinates them, is the target of autoantibodies in early RA. This suggests a model to explain the production of ACPAs in the absence of T cells to citrullinated proteins. ACPAs could arise because, at first, PADs are recognised by T cells, which, in turn help the production of autoantibodies to proteins being citrullinated by

PADs, through a hapten/carrier model. Here, we test this model in mice.
Methods HLA-DRB1*04:01 transgenic mice were immunised subcutaneously with PADs or phosphate buffered saline (PBS) in Freund's complete adjuvant (CFA). Three booster injections of PAD or PBS in Freund' incomplete adjuvant (IFA) were given subcutaneously 15, 35 and 55 days later. Mice were:
1. tested for anti-PAD antibodies by ELISA.
2. tested for T cell responses to PADs, native or citrullinated fibrinogen 65 days after PAD immunisation.
3. tested for anti-citrullinated fibrinogen antibodies by ELISA using fibrinogen peptides under citrullinated and native form.

Results HLA-DRB1*04:01 transgenic mice immunised with PADs developed antibodies and T cells to PADs and IgG antibodies to citrullinated peptides from fibrinogen, in the absence of T cell response to native or citrullinated fibrinogen.
Conclusions T cell immunisation to PAD proteins triggers ACPAs through a hapten carrier mechanism in which the carrier is PAD which performs citrullination and the hapten any protein being citrullinated by PAD.
Disclosure of interest None declared

P013 PORPHYROMONAS GINGIVALIS INFECTION LINKED TO RA ONSET AND ANTI TNF ALPHA TREATMENT NON-RESPONSE

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Introduction Differences in enzymatic activity and pathogenic impact of *Porphyromonas gingivalis* (*P.g.*) peptidylarginine deiminase (PPAD) for development of RA have been published, confounded by different PPAD variations and methods used.
Objectives Enzymatic active PPAD isolated from an RA patient (RA-PPAD) was first time linked to citrullination of RA autoantigens, diagnosis, therapy response and RA-onset.
Methods Recombinant RA-PPAD cloned, verified by DNA sequencing and expressed in *Escherichia coli and purified*. RA-PPAD and its enzymatic activity was analysed using 2D-Elektrophoresis, mass spectrometry (MS), immunoblot and ELISA.
Results RA-PPAD autocitrullinates amino acid position 63 (aa_{63}) and exhibits so far two new amino acid mutations aa_{73} F to L and aa_{447} E to V. Anti-citrullinated RA-PPAD antibodies were detected in 38% (n=36) of patients with RA, but were absent in Systemic lupus erythematosus (n=30), Osteoarthritis (n=36) and control sera (n=23). Twenty percent of RA patients (n=30) showed an increase in antibody-titre against citrullinated RA-PPAD after RA onset. High antibody titre against the cit-PPAD-peptide of 15aa (CPP) derived from the autocitrullination site (R_{63}) correlates with TNFα-inhibitor (TBA) non-response (n=61). Anti-CPP levels correlate with DAS28,rheumatoid factor, α-CCP 2 levels and increase with age. RA-PPAD is able to citrullinate internal arginines in fibrinogen, vimentin, hnRNP-A2/B1 and histone H1. This internal citrullination-sites are recognised by RA sera and able to bind HLA401.
Conclusions Failure of *P.g.* clearance in RA patients may lead to excessive exposure of citrullinated self-antigens and bacterial antigens inducing immune-mimicry. *P.g.* infection can be linked to RA and its correlation to TBA non-response leads to the suggestion to clear *P.g* infection before α-TNF treatment.
Disclosure of interest None declared

P014 FAM167A/BLK IS A SUSCEPTIBILITY LOCUS IN AUTOIMMUNE DISEASES: CHARACTERISATION OF THE FAM167 GENE FAMILY

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Introduction Genome-wide association studies of multiple autoimmune diseases, including Sjögren's syndrome, systemic lupus erythematosus and rheumatoid arthritis have identified associations in the *FAM167A/BLK* locus. eQTL (expression quantitative loci) analyses in peripheral blood cells show a significantly increased expression of *FAM167A* for the disease associated genotypes. While *BLK* acts downstream of the B cell receptor, the function of *FAM167A* is unknown. Additionally, the role of the only homologous protein, its gene family member *FAM167B*, remains unexplored.
Objectives To elucidate the potential role of these proteins in susceptibility, or pathogenesis of autoimmune disease, we aim to characterise the *FAM167* gene family by both bioinformatic and experimental approaches.
Methods Public databases, including NCBI, GEO, Uniprot, CCLE, PhylomeDB were searched for information on the *FAM167* gene family and its two members. The FAM167 protein sequences were analysed with structure prediction tools including YASPIN and D2P2 server. The mRNA levels of the genes of interest were determined by qPCR analysis on organs from 12 week old C57BL/6 mice and cells of selected human cancer cell lines.
Results After protein sequence analysis we found that the *FAM167* gene family shows no homology to any other annotated genes but is highly conserved in vertebrates. The FAM167A and FAM167B proteins don't comprise any previously known protein domains and are predicted to contain both helical and disordered protein structures. Based on the analysis of their protein structure prediction and their conservation we propose that the FAM167 proteins contain two distinct modules linked by a variable linker accounting for the different size of 24 and 18 kDa for FAM167A and B, respectively. Database research revealed that *FAM167A* is expressed in many cancer cell lines whereas *FAM167B* shows a specifically high expression only within melanoma lines according to CCLE. *FAM167A* expression was confirmed for the myeloma cell line LP1 and the monocytic cell line THP1 experimentally. High *FAM167B* expression was found in the melanoma cell line SKMEL28. Expression profiling of mouse organs collected from C57BL/6 mice revealed high expression of *Fam167a* in lung, spleen, skeletal muscle and brain. *Fam167b* had its peak expression in adrenal glands, kidney and liver, with low or no expression observed in other analysed organs.
Conclusions The *FAM167A/B* genes show distinct expression profiles both at the organ and the cellular level. Further *in vitro* and *in vivo* studies will be implemented to unravel the function of this gene family.
Disclosure of interest None declared