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 versus 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P010 ABSTRACT WITHDRAWN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 SYNOVIAL TISSUE AT ONSET OF CLINICAL RHEUMATOID ARTHRITIS

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 uninfamed 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 TCRß 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 TCRß sequence.3 For each sample, 3,570 TCRß sequences were analysed.

Results During the at-risk phase the TCRß 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 TCRß 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 TCRß 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


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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

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
Porphryromonas gingivalis infection linked to RA onset and anti TNF alpha treatment non-response

Methods HLA-DRB1*04:01 transgenic mice were immunised subcutaneously with PAs 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 PAs, 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 PAs developed antibodies and T cells to PAs 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

FAM167A/BLK is a susceptibility locus in autoimmune diseases: characterisation of the FAM167 gene family

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