Results: The proteome analysis of SF allowed deepest proteome analysis so far (Proteoforms >1300) as well as a number of citrullinated sites. The three patient groups could be differentiated by cluster analysis and the occupancy of each modified site calculated. The investigation of the intestinal tissue enabled identification of 223 citrullinated peptides from 121 proteins. Three of the peptides were unique to RA. The list of citrullinated proteins was enriched in extracellular and membrane proteins and included known targets of anticitrullinated protein antibodies (ACPAs). Investigation of collagen-induced arthritis mouse model enabled differential analysis of the proteome as response to treatment and determination of the PTMs associated with this model.
Conclusions: Our deep proteome based analysis of tissue and biofluids have enabled an extended catalogue of citrullinated proteins and sites relevant to improved disease subtyping as well as a source of citrullinated sites for future studies.

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SAT0021 HUMAN CD4 T CELLS AND SYNOVIAL FIBROBLASTS COOPERATE TO PROMOTE INFLAMMATION IN THE RA SYNOVIAL JOINT

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by synovial tissue proliferation and degradation of articular cartilage. Activated synovial fibroblasts proliferate and express matrix-degrading proteases, adhesion molecules and proinflammatory cytokines, which contribute to cartilage and joint destruction. Moreover, synovial cell activation correlates with infiltration of inflammatory lymphocytes and monocytes which in turn contribute to synoviocyte activation, thus further exacerbating inflammation.
Objectives: The functional relationship linking fibroblasts and T lymphocytes in this complex microenvironment has yet to be characterised. Therefore, we established an *in vitro* model to examine the outcomes of co-culturing activated human CD4 T cells with RA synovial fibroblasts.
Methods: Co-culture assays were carried out using immortalised K4IM RA synovial fibroblasts or synovial fibroblast cells derived from arthroscopy biopsies of RA patients. Human CD4 T cells were stained with a proliferation-tracking dye and co-cultured with pre-seeded synovial fibroblasts for 5 days. The resulting cell cultures and supernatants were examined for proliferation, cytokine production, secretion of matrix metalloproteinases and expression of adhesion molecules.
Results: We found that CD4 T cells and K4IM cells reciprocally induced an increased expression of adhesion molecules ICAM and VCAM. Furthermore, co-culture of CD4 T cells and synovial fibroblasts resulted in proliferation of CD4 T cells expressing increased levels of the proinflammatory cytokines IFN-γ and IL-17a and RANKL after 5 days. Lastly, co-culture of T cells and synovial fibroblasts resulted in secretion of IL-6, IL-8, IFN-γ and IL-17a and matrix metalloproteinases MMP-1 and MMP-3.
Conclusions: These results indicate that CD4 T cells work mutually with synoviocytes to create an inflammatory microenvironment likely to promote joint destruction. Future studies will characterise the role of glucose metabolism in these cells and investigate if metabolism is intrinsically coupled to effector functions in these cells.
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SAT0022 EPIGENOME-WIDE ASSOCIATION STUDY OF RHEUMATOID ARTHRITIS IDENTIFIES DIFFERENTIALLY METHYLATED LOCI IN B CELLS

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Background: Epigenetic regulation of immune cell types could be critical for the development and maintenance of autoimmune diseases like Rheumatoid Arthritis (RA). B cells are highly relevant in RA, since patients express autoantibodies and depleting this cell type is a successful therapeutic approach. Epigenetic variation, such as DNA methylation, may mediate the pathogenic activity of B cells.
Objectives: In this study, we performed an epigenome-wide association study (EWAS) for RA with three different replication cohorts, to identify disease-specific alterations in DNA methylation in B cells.
Methods: Genomic methylation in isolated B lymphocytes was assayed on the Illumina HumanMethylation450 BeadChip, assaying >450,000 different CpG sites. Differential methylated positions (DMPs) were identified in a discovery cohort using a single-point analysis using logistic regression, as well as a pathway-level analysis using a newly developed permutation-based method. A discovery cohort of 50 RA patients and 75 healthy controls from Spain was used to identify the most differentially methylated regions after multiple test correction. Using an independent sample of 15 patients and 15 controls from the same population we performed a replication analysis of the most significant CpG sites and pathways. Using an additional case-control sample of 24 individuals from the UK we provided further evidence of association of the DMPs with RA. Finally, in silico datta from a cohort of systemic lupus erythematosus patients (SLE, n=47) and controls (n=56) from the US, we tested the association of the associated DMPs.
Results: A total of 64 CpG sites were found to be differentially methylated in RA patients compared to controls in the discovery stage after multiple test correction ($q<0.05$). Six biological pathways were also differentially methylated in RA B cells. Analysis of these epigenetic changes in the independent Spain cohort replicated the association of 10 CpG sites located on 8 genes and 2 intergenic regions. Differential methylation at the *CBL* signaling pathway was also replicated. Using the UK case-control cohort, association between RA risk and methylation levels at *CD1C* (P=2.26x10^{-9}) and *TNFSF10* (P=1.67x10^{-8}) loci was further validated. Most of the replicated DMPs associated with RA were also found to be associated with differential methylation in SLE B cells.
Conclusions: Our results highlight genes that may drive the pathogenic activity of B cells in RA and suggest shared methylation patterns with SLE.
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SAT0023 DIRECT-ACTING ANTIVIRAL-BASED THERAPY RESTORES IMMUNE TOLERANCE IN HEPATITIS C-INDUCED CRYOGLOBULINEMIA VASCULITIS

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Objectives: Interferon-free direct-acting antiviral (DAA)-based therapy has proven to be very effective in patients with hepatitis C virus-cryoglobulinemia vasculitis (HCV-CV). However, their mechanisms of action and their effects on cellular immunity remain poorly defined.
Methods: 27 HCV-CV patients treated with DAA therapy, 12 healthy donors (HD) and 12 HCV were included. We investigated the effects of DAA-based therapy on cellular and cytokine abnormalities in HCV-CV patients by flow cytometry, cytokine Multiplex and enzyme-linked immunosorbent assay.
Results: Compared with HD and HCV, pre-DAA abnormalities in HCV-CV patients included a decreased percentage of $CD4^+CD25^{hi}FoxP3^+$ regulatory T cells ($P<0.01$) with increases in $IgM^+CD21^{-/low}$ memory B cells ($P<0.05$), $CD4^+IFN\gamma^+$ ($P<0.01$), $CD4^+IL17A^+$ ($P<0.01$) and $CD4^+CXCR5^+IL21^+$ follicular helper T cells (Tfh) ($P<0.01$). $IgM^+CD21^{-/low}$ memory B cells were negatively correlated with regulatory T cells (Tregs) (P=0.03), and positively correlated with Tfh (P=0.03) and serum cryoglobulin levels (P=0.01). DAA-based therapy was associated with an increase in Tregs frequency (1.5% ± 0.18% versus 2.1% ± 0.18%), and decreased $IgM^+CD21^{-/low}$ memory B cells and Tfh percentage (35.7% ± 6.1% versus 14.9% ± 3.8%, and 12% ± 1.3% versus 8% ± 0.9%, respectively). B lymphocyte stimulator receptor 3 and programmed death-ligand 1 staining expression on B cells increased in HCV-CV after DAA-based therapy (MFI 37±2.4 versus 47±2.6, $P<0.01$; and 29±7.3 versus 48±9.3, $P<0.05$ respectively).
Conclusions: Our results indicate that DAA-based therapy effectively normalizes many of the disturbances in peripheral B and T lymphocyte homeostasis of HCV-CV patients.
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SAT0024 ALTERATIONS OF PERIPHERAL BLOOD B-CELL SUBSETS IN EARLY RHEUMATOID ARTHRITIS

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Background: Alterations of B-cell subpopulation have been described in the