OP0191

EPIGENETIC PROFILING OF SYNOVIAL FIBROBLASTS REVEALS STRUCTURAL DNA DYNAMICS AT DISEASE IMPLICATED CHROMOSOME REGIONS

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Background: One of the more striking findings from genome-wide association studies (GWAS) is how the majority of disease associated genetic variants are found within gene regulatory regions, known as enhancers. It is now well established that these enhancers regulate target genes through physical interactions. We, and others, have shown that these interactions can vary between cell types and act over long distances. Therefore, if we are to fully translate GWAS findings, we need link disease associated enhancers to their target genes, in the relevant cell types. So far we only have a limited picture in rheumatoid arthritis (RA) relevant cell types, since synovial fibroblasts (SF) have been omitted from this type of chromatin interaction analysis.

Objectives: Investigate dynamic Hi-C interactions in unstimulated and stimulated SF, mapping RA enhancers to their target genes, and correlating with gene expression.

Methods: We cultured SF from synovial tissues of RA patients. After stimulation of the SF (n=6) with 10 ng/ml TNF for 24h, Hi-C and RNA libraries were generated and sequenced on the Illumina HiSeq 4000. We called dynamic active and inactive regions of the genome (A/B compartments), mapped on RA associated enhancers, linked these to target genes and correlated the interactions with dynamic expression.

Results: We found a region on the chromosome 6q23, intergenic between *OLIG3* and *TNFAIP3*, with an enhancer containing SNPs associated with RA to be dynamically linked to the *TNFAIP3* gene through DNA activity, interactions and corresponding gene expression.

As shown in figure 1a, the genomic region containing the RA associated variants (red square) only resides in an open, active region of DNA (black bar, compartment A) upon TNF-stimulation of the SF. This region then makes a strong interaction with the promoter of *TNFAIP3* (figure 1a) in stimulated cells, which corresponds to a more than 100-fold increase in *TNFAIP3* gene expression (figure 1b).

Conclusion: Using independent data, we have indicated how the RA region on 6q23 could influence the expression of *TNFAIP3*, under stimulatory conditions in SF cells. This supports previous work, where this particular 6q23 enhancer is preferentially active in RA synovium, compared to osteoarthritis, reflecting the different stimulatory conditions in each disease (Ai *et al.* 2018). We have successfully linked an RA associated enhancer to its target gene in SF, indicating how the enhancer works over a large distance to interact with and regulate the *TNFAIP3* gene. Furthermore we show that this enhancer containing RA associated SNPs is only active upon stimulation with TNF in SF.

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Figure 1a

Figure 1b

Disclosure of Interests: None declared DOI: 10.1136/annrheumdis-2019-eular.4094

OP0192

DISSECTING THE LONG-RANGE GENE REGULATION OF RHEUMATOID ARTHRITIS RISK ENHANCERS AT THE 5Q11 LOCUS USING THE COMPLEMENTARY APPROACHES OF CRISPRa AND CRISPRi

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Background: Large-scale genome wide association studies (GWAS) in rheumatoid arthritis (RA) have revealed that the majority of associated risk variants lie in non-coding regions of the genome. For RA it has been demonstrated that risk variants are significantly enriched in T cell specific enhancers. One such susceptibility locus known as 5q11 is the third strongest association with RA. At this locus the lead risk variants lie in two distinct active enhancer elements intronic to the *ANKRD55* gene. Previous work using chromatin conformation has shown these enhancers make physical contact with the promoter of *IL6ST*, 150 kb away on the linear chromosome¹. Whilst *ANKRD55* is a gene of unknown function, *IL6ST* is part of the IL6R complex which regulates the IL6 signalling pathway. Tocilizumab is already an approved drug for RA, which works against the IL6R. CRISPR is an ideal tool to dissect this enhancer-gene relationship to provide us with empirical evidence that the intronic enhancer within *ANKRD55* regulates the expression of *IL6ST*. Understanding how RA risk genetic background affects gene regulation will give us a better understanding of the underlying biology of the disease and can ultimately be used to help repurpose or discover novel drug therapies.

Objectives: To use CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) to perturb the *ANKRD55* intronic enhancer and measure the downstream effect on the expression of *IL31RA*, *IL6ST*, *ANKRD55*.

Methods: We used dead (dCas9) a modified CRISPR Cas9 enzyme that is catalytically inactive, that precisely targets DNA, but does not cut. Instead, effector molecules are fused to the dCas9 (VP64 for activation and KRAB for repression) to alter the transcription of the targeted genes. Two lead functional risk SNPs at the 5q11 risk locus rs10065637 and rs7731626 ($R^2 = 0.4472$) lie in two distinct enhancer elements separated by 5.5kb. We designed 4 guides across each enhancer, and transduced a T cell line (Jurkat) using lentiviral delivery; first dCas9-KRAB and dCas9-VP64 and subsequently the pool of guides for each enhancer alongside controls. We cultured the cells until confluent and then sorted the top 60% expression of BFP-GFP (KRAB) or MCherry-GFP (VP64). RNA was extracted and then a qPCR was performed (Quantstudio) for *IL31RA*, *IL6ST* and *ANKRD55*.

Results: In the CRISPRa Jurkat cells the guides targeted to both SNP containing enhancers significantly increased the expression of *ANKRD55*: rs7731626 ($p < 0.0001$) and rs6859219 ($p = 0.0003$) respectively. They also increased the expression of *IL6ST* rs7731626 ($p = 0.0023$) and rs6859219 ($p = 0.01$). In CRISPRi Jurkat cells the guides targeted to both SNP containing enhancers significantly decreased the expression of *ANKRD55*: rs7731626 ($p < 0.001$) and rs6859219 ($p = 0.0071$). When guides were simultaneously used for both enhancers rs6859219+rs7731626 there was also a significant decrease in expression of *ANKRD55*. ($p < 0.0001$). Only the pool guides against both enhancers significantly decreased the expression of *IL6ST* ($p < 0.0001$). In both systems no significant effect was seen on the expression of *IL31RA* (Figure 1).

Figure 1

Conclusion: Results from CRISPRi and CRISPRa are concordant and support the idea that intronic enhancers within *ANKRD55*, containing SNPs associated with RA susceptibility, can regulate the expression of both *ANKRD55* and also modulate long range gene regulation with *IL6ST* to influence disease risk. These techniques are crucial to begin to dissect and translate GWAS findings.

REFERENCE:

- [1] Capture Hi-C reveals novel candidate genes and complex long-range interactions.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.7946

OP0193 CAMP RESPONSE ELEMENT MODULATOR (CREM) α INDUCES DUAL SPECIFICITY PROTEIN PHOSPHATASE (DUSP)4 THROUGH EPIGENETIC REMODELING, PROMOTING IL-17A AND REDUCING IL-2 EXPRESSION IN T CELLS

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Background: Tissue inflammation and organ damage in systemic lupus erythematosus (SLE) have been linked to effector T cells that are characterized by increased IL-17A and reduced IL-2 production(1). T cells from patients with SLE express increased levels of the transcription factor cAMP response element modulator (CREM) α that contributes to altered cytokine expression(1-3). However, the exact molecular events contributing to dysregulated IL-17A and IL-2 expression are incompletely understood.

Objectives: To investigate molecular events that promote effector T cells in health and disease. The definition of molecular regulators of effector T cell generation and activity may deliver new biomarkers and potential therapeutic targets in disorders characterized by altered effector T cell function, including (but not limited to) SLE.

Methods: Using CRISPR/Cas9 genome editing and lentiviral transduction, we generated CREM α deficient or overexpressing Jurkat T cells. Gene expression profiles in Jurkat and primary human CD4⁺ T cells were assessed by qRT-PCR and mRNA probe-based hybridization techniques. Gene regulation events were investigated using luciferase reporter assays (*trans*-activation) and ChIP. Interactions between CREM α and the transcriptional co-activator p300 were assessed using proximity ligation assays and p300 knock-down with siRNAs.

Results: We link CREM α production in effector CD4⁺ T cells with increased expression of dual specificity protein phosphatase (DUSP)4. Using genetically modified Jurkat T cells, we demonstrate that CREM α induces DUSP4 through recruitment of the transcriptional co-activator p300 and histone H3K18 acetylation. Using DUSP4 transfection models and genetically modified Jurkat T cells, we support previous reports suggesting that DUSP4 induces IL-17A while limiting IL-2 expression. Furthermore, we demonstrate that CD4⁺ T cells from patients with juvenile-onset SLE share the phenotype with CREM α over-expressing CD4⁺ T cells with increased DUSP4 expression that contributes to imbalanced IL-17A and IL-2 production.

Conclusion: Collectively, our results deliver previously unknown CREM α -mediated molecular mechanisms promoting effector T cells and support the central involvement of CREM α in the pathophysiology of SLE. CREM α and DUSP4 may prove valuable as disease biomarkers and/or targets in the search for individualized and target-directed treatments.

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Acknowledgement: This is a summary of independent research funded by the Fritz-Thyssen-Foundation and carried out at the National Institute of Health Research (NIHR) Alder Hey Clinical Research Facility. The views expressed are those of the authors.

Disclosure of Interests: Sigrun Hofmann: None declared, Katrin Maebert: None declared, Franz Kapplusch: None declared, Susanne Russ: None declared, Christian Hedrich Grant/research support from: Novartis Pharmaceuticals for

Research study on effector T cells in psoriasis, Speakers bureau: In 2016: Roche Pharmaceuticals, RheumatoLogisch, Dresden, Germany; Novartis, Advisory board > Travel costs.

DOI: 10.1136/annrheumdis-2019-eular.999

OP0194 HISTONE DEACETYLASE 1 (HDAC1): A KEY MEDIATOR OF T CELLS FOR THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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Background: Despite enormous efforts to develop new therapeutic strategies for treatment of rheumatoid arthritis (RA), the large number of non responding patients to currently available drugs underlies the unmet need to identify new therapeutic targets. Certain CD4⁺ T cell subsets, especially those polarized toward the T helper (Th) subsets Th1 and Th17, have been shown to be major drivers of inflammation in patients with RA. The expression of their key transcription factors is controlled by histone modifications which includes acetylation of *lysine residues* mediated by histone deacetylases (HDAC). Indeed, pan HDAC inhibitors have been shown to be a potential therapeutic strategy. However, major side effects limited the clinical use and underline the need of more specific HDAC inhibitors.

Objectives: We addressed the individual role of HDAC1 on the development of collagen-induced arthritis model (CIA), which partially reflects human RA.

Methods: Mice with a T cell specific deletion of HDAC1 (HDAC1 cKO) were generated by using the CD4Cre/LoxP system. At week 8 of age arthritis was induced in wild type (WT) and HDAC1 cKO mice by immunizing with chicken collagen II (CII), emulsified in complete Freund's adjuvant. After 21 days mice received a booster injection of CII. The animals were 3 times per week scored for paw swelling and grip strength. Anti-CII antibody levels were determined by ELISA. Various cell subsets, including Th cells, were detected in the blood, the spleen and the draining lymph node by FACS analysis. To test antigen-specific T cell activation we performed *in vitro* restimulation of spleen and lymph node cells with collagen II followed by assessment of cytokine production and quantification of the proliferation rate using 3Hthymidine incorporation.

After 4 and after 10 weeks mice were sacrificed and paraffin sections of the affected joints were analyzed for histomorphologic signs of inflammation, cartilage and bone destruction.

Results: Eighty percent of the animals developed serum anti-CII antibodies (IgM and IgG) whereby the antibody levels were a day 21 of disease similar between the HDAC1 cKO and the WT mice. Furthermore, no differences in the production of antibody subclasses, especially of pathogenic IgG2c antibodies, were observed. Enhanced percentages of Th1 and Th17 cells among HDAC1-null CD4⁺ T cells were detected after immunization in the HDAC1 cKO mice. Nonetheless and unexpectedly, these mice did not develop any signs of disease at the clinical level while WT mice developed pronounced paw swelling and loss of grip strength. In accordance with the clinical data, histological analysis revealed no signs of inflammation, no bone erosion and no appearance of osteoclasts in the joints of HDAC1 cKO mice. This appeared to be mainly caused by an impaired migratory capacity of HDAC1 cKO CD4⁺ T cells, so that they were unable to invade the joints.

Conclusion: Our data show the importance of HDAC1 as a key immune regulator in the pathogenesis of T cell driven collagen induced arthritis. Therefore it might be considered as an interesting novel therapeutic target in RA.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.5619

OP0195 SELECTIVE EXPANSION OF REGULATORY T-CELLS IN HUMANS BY A NOVEL IL-2 CONJUGATE T-REG STIMULATOR, NKTR-358, BEING DEVELOPED FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Background: Impaired IL-2 production and dysfunction of regulatory T cells (Tregs) have been identified as key immunological defects leading to the