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.

REFERENCE:
CAMP RESPONSE ELEMENT MODULATOR (CREM) A
Brian Kotzin

HISTONE DEACETYLASE 1 (HDAC1): A KEY SELECTIVE EXPANSION OF REGULATORY T-CELLS
REFERENCES:


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Disclosure of Interests: None declared


CAMP RESPONSE ELEMENT MODULATOR (CREM) 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)iso that contributes to altered cytokine expression(1-3). However, the exact molecular events contributing to dysregulated IL17A and IL2 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 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 CREMiso and the transcriptional co-activator p300 were assessed using proximity ligation assays and p300 knock-down with siRNAs.

Results: We link CREMiso 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 CREMiso induces DUSP4 through transcription 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 CREMiso-over-expressing CD4+ T cells, and DUSP4 expression that contributes to imbalanced IL-17A and IL-2 production.

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

REFERENCES:


Disclosure of Interests: None declared


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) subs 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, where 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.

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, cartilage and bone destruction.

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


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

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