validation including animal models, it may even offer a novel therapeutic tool for AIDs and T-ALL.

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P141 DIFFERENTIAL DNA METHYLATION IN PERIPHERAL NAÏVE CD4+T-CELLS IN EARLY RHEUMATOID ARTHRITIS PATIENTS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Alterations in DNA methylation patterns (epimutations) have been related to several diseases, including Rheumatoid Arthritis (RA). We hypothesise that such epimutations occur early in the RA disease process. T-cells are important cells in early pathogenesis, therefore we choose to analyse patterns of differential methylation (DM) of the DNA of CD4+ T cells.

Objectives To identify changes in DNA methylation pattern of naïve CD4 +T cells in early, drug naïve RA patients, towards understanding early events in disease pathogenesis.

Methods DNA methylation of 480,000 CpGs (Illumina methylation genome-wide array) were analysed in cell sorted, naïve CD4+ T cell from 6 healthy control (HC) and 10 RA patients. To priorities DM gene, we designed a scoring system.¹ We examined potential interactions between DM genes using the STRING-database of protein-protein network analysis. Flow cytometry was used to characterise subpopulations of naïve CD4+ T cells in early RA for 4 DM genes, coding for cell surface molecules.

Results We observed 648 DM-genes in naïve T-cells in early RA (354 hypo and 294 hyper-methylated). STRING analysis of these 648 genes/proteins revealed central of JAK1/STATs signalling, associated with an IL6/IL6R/STAT3 signalling node, a 2nd node for STAT2 linked to downstream interferon signalling, a 3rd node centred on IL2/IL15/STAT5, and many other associations notably related to TNF-alpha and TGF-beta signalling as well as a Th17 cell differentiation axis. Several genes were coding for cell surface proteins, notable CD4, IL2R, IL6R and CD62L. Analysis of CD4 and IL2R confirmed significant increased expression between RA and HC. CD62L identified 2 subpopulations of naïve T-cell in RA the classical naïve CD62L+ cells and CD62L- naïve T-cells as previously reported². IL6R expression was reduced in CD62L- naïve CD4+ T cell compared to CD62L+ cells.

Conclusions DNA methylation pattern in naïve CD4+ T cell point to a central role for JAK1/STAT3/IL6 signalling in very early RA pathogenesis, with a pivotal role in diversification towards other pathways (notably TNF, Th17, Th1), which

may already contribute to patient heterogeneity at this stage. Our data provides more understanding of early RA pathogenesis and should facilitate novel therapeutic strategies by targeting the right pathways.

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Disclosure of Interest None declared.

P142 ROS101, A NOVEL TARGETED METHOTREXATE PRODRUG, SELECTIVELY ACTIVATED BY REACTIVE OXYGEN SPECIES (ROS) SHOWS REDUCED TOXICITY IN RAT CIA MODEL OF RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction The use of methotrexate (MTX) for the treatment of rheumatoid arthritis (RA) is limited by serious adverse effects. Some effects, such as stomatitis and gastric ulcer, can be alleviated by folate supplement. Others are folate-independent, e.g. fibrosis of liver and lung. Moreover, MTX further elevates the micronuclei count, which is generally already increased in RA.

In RA, inflamed tissue is characterized by up to 100-fold increased concentrations of reactive oxygen species (ROS, including hydrogen peroxide) compared to healthy tissue.

A novel MTX prodrug, ROS101 in development for the treatment of RA, releases MTX at exposure to ROS. This restricts MTX exposure to target tissues with increased ROS levels, e.g. the synovial membrane in RA.

Objectives To investigate the effect and toxicity of a targeted MTX prodrug in a rat CIA model of rheumatoid arthritis.

Methods ROS101 was tested in a collagen induced arthritis (CIA) model in dark agouti (DA) rats (10 per group) against vehicle and 0.3 mg MTX i.p./day at equimolar doses. Arthritis was induced by s.c. injection of an emulsion of collagen type II in complete Freund's adjuvant. Treatment was initiated at a mean arthritis severity score of 2/60. Blinded disease evaluation took place 3 times/week from day 10. Bone marrow cells for micronucleus test were obtained from the femur, pictures captured with LARS.4.8 software and analyzed blinded. Individual rat data were included until termination.

Results Mean and maximum arthritis severity score was reduced in rats in the ROS101 group compared to vehicle (mean score 6.3±3.1 vs. 14.1±3.3, mean±SEM); (max score 12.6±5.3 vs. 32.3±5.3, mean±SEM, p<0.05). All rats in the ROS101 group completed the study until day 32. Reduced arthritis severity in the MTX group after day 22 was accompanied by overt toxicity, causing all rats to be terminated from day 24 to 29. There was no significant difference in body weight between rats in the ROS101 and vehicle groups, whereas body weight in the MTX group decreased significantly from day 22 due to toxicity, including GI effects. Bone marrow micronucleus count was reduced in the ROS101

group in contrast to the MTX group, where micronucleus count was elevated as compared to the vehicle control group.

Conclusions The CIA study in rats indicates that the MTX prodrug ROS101 may be efficacious for the treatment of RA at an equimolar dose compared to MTX, while avoiding adverse effects known to restrict treatment with MTX.

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EFFECT OF LOW-DOSE IONIZING RADIATION ON THE INFLAMMATORY PHENOTYPE OF ADIPOCYTES AND DIFFERENTIATION OF OSTEOCLASTS (IN VITRO)

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Adipose tissue is a complex endocrine organ that produces a variety of immune and inflammatory mediators. Adipocytes, the dominant cell type of adipose tissue, are known to support inflammatory processes in musculoskeletal diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) by release of different cytokines and adipokines. During this process, osteoclastogenesis is also enhanced and results in an imbalance of bone metabolism. Low-dose radiation therapy (LD-RT) is known to attenuate inflammation and to increase the mobility of patients suffering from RA or OA.¹

Objectives In our previous work, we observed a decrease of visfatin levels in serum of patients treated with low-dose ionizing radiation during exposure to the alpha-emitter radon.² In the same study, a decrease of markers for bone resorption after radon exposure was detected. Based on this, we next compared the response of human adipocytes derived from subcutaneous and infrapatellar adipose tissue to ionizing radiation with respect to release of adipokines and other inflammatory factors (IL-6, IL-8). In parallel, we analyzed the effect of ionizing radiation on differentiation capacity of osteoclast (OC) precursors into mature, bone resorbing OC.

Methods Human subcutaneous preadipocytes and human infrapatellar preadipocytes were irradiated with different doses of ionizing radiation, and release of inflammatory factors was measured in the cell culture supernatants using ELISA. OC precursors were isolated from human donor blood, differentiated according to standard protocols and analyzed by fluorescent stainings for cell nuclei, tartrate-resistant acidic phosphatase (TRAP) and actin filaments.

Results The results revealed that the release of adipokines and inflammatory cytokines (IL-6, IL-8) was not significantly affected by ionizing radiation. Further, it was found that differentiation of OC precursor cells into mature OC is reduced after irradiation.

Conclusions The observations made in this study suggest that adipocytes are probably not the main source of modified adipokine levels in the arthritic joint. However, an observed

tendency of adipocytes to increase fat accumulation after irradiation suggests radiation-induced changes in functionality of human adipocytes which could have an indirect impact on the radiation response of the tissue. OC respond to radiation by reduced differentiation and structural changes, but the impact on functionality needs to be further tested.

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HCQ ALLEVIATES 5-FU-INDUCED INTESTINAL INFLAMMATION THROUGH INHIBITING TLR9-DEPENDENT DNA SENSING PATHWAY

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Career situation of first and presenting author Young investigator.

Introduction Evidences revealed that chemotherapies could trigger DNA release, then induce inflammation of intestinal tissues which damp the effect of anti-cancer treatment.^{1 2} DNA released induces the translocation of TLR9 to endolysosomes and subsequent nuclear factor-κB (NF-κB) activation, which leads to interleukin-1β (IL-1β) secretion and inflammation.³ Targeting TLR9-dependent DNA sensing pathway may be a valuable therapy for chemotherapy induced intestinal mucositis.

Objectives This study aims to investigate whether hydroxychloroquine (HCQ), suppresses 5-FU-induced intestinal mucositis through inhibiting TLR9-dependent DNA sensing pathway.

Methods The effect of HCQ on 5-FU-induced intestinal mucositis were examined *in vivo* and *in vitro*. We established 5-FU-induced intestinal mucositis model and assessed body weight, diarrhea score and histopathologic changes following HCQ treatment *in vivo*, then TLR9 and NF-κB expression of small intestine and IL-1β secretion of serum were analyzed. Bone marrow-derived macrophages (BMDMs) were cultured, transfected with calf-thymus DNA(CT-DNA) and treated with HCQ for 6 hour *in vitro*. TLR9 and NF-κB expression and IL-1β secretion in BMDMs were then investigated.

Results HCQ treatment markedly attenuated body weight loss, severity of diarrhea, intestine shortening, and destruction of small intestine in histopathology of 5-FU- treated mice *in vivo*. Also HCQ treatment inhibited TLR9 and NF-κB expression in small intestine of 5-FU- treated mice and pro-inflammatory IL-1β secretion in serum of 5-FU- treated mice. Meanwhile administration of HCQ reduced the number of macrophages in small intestine of 5-FU-treated mice. Then BMDMs were cultured, transfected with CT-DNA and treated with HCQ *in vitro*. We found HCQ efficiently inhibited TLR9 expression, translocation of NF-κB to nucleus, and IL-1β secretion in supernatant of CT-DNA stimulated BMDMs.

Conclusions These results provides a new insight into the mechanism of chemotherapy induced intestinal mucositis and