Differential DNA Methylation in Peripheral Naïve CD4+ T-Cells in Early Rheumatoid Arthritis Patients

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Introduction Alterations in DNA methylation patterns (epimutations) have been related to several diseases, including Rheumatoid Arthritis (RA). We hypothesise that such epimutations may 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.

Methods DNA methylation of 480,000 CpGs (Illumina methylation genome-wide array) were analysed in cell sorted, naïve CD4+ T cells from 6 healthy control (HC) and 10 RA patients. To priorities DM gene, we designed a scoring system including animal models, it may even offer a novel therapeutic tool for AIDs and T-ALL.

References

Acknowledgements
We would like to thanks Prof. S. Savvides, Dr. K. Verstraete (VIB, Belgium) and Dr. Joao Barata (IMM, Portugal) for their contribution on this work.

Disclosure of Interest
None declared.

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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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.

Results DNA methylation patterns 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.

Disclosure of Interest
None declared.

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

References
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 prodruk 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.

Acknowledgements C.A. Hansen, co-founder of ROS Therapeutics.


P144 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.

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. 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 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 staining 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.

REFERENCES

Acknowledgements German Federal Ministry of Education and Research (02NUK017A).

Disclosure of Interest None declared.

P145 HCQ ALLEVIATES 5-FU-INDUCED INTESTINAL INFLAMMATION THROUGH INHIBITING TLR9-DEPENDENT DNA SENSING PATHWAY

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10.1136/annrheumdis-2018-EWRR2019.128

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. DNA released induces the translocation of TLR9 to endolysosomes and subsequent nuclear factor-kB (NF-kB) activation, which leads to interleukin-1β (IL-1β) secretion and inflammation. 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-kB 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-kB 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 intestinal in histopathology of 5-FU-treated mice in vivo. Also HCQ treatment inhibited TLR9 and NF-kB 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-kB 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