P148 JAK-INHIBITION BY BARICITINIB AND TOFACITINIB AMELIORATES PATHOLOGICAL BONE LOSS

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

Introduction Many cytokines relevant to rheumatoid arthritis (RA) rely on the janus kinase – signal transducer and activator of transcription (JAK-STAT) signaling pathway. The JAK-inhibitors tofacitinib and baricitinib, targeting JAK3/JAK1 and JAK1/JAK2 respectively, have been approved for treatment of RA¹. While currently available therapies reduce inflammation, erosive damage in involved joints is still irreversible. However, preliminary data suggests an influence of JAK inhibition on local bone healing.

Objectives To study the role of JAK-inhibition in osteoblast and osteoclast-mediated bone homeostasis and its capacity to alleviate structural bone damage *in vivo*.

Methods For steady state analysis C57BL/6 (WT) mice received tofacitinib QD by oral gavage for 6 weeks. WT mice of the ovariectomy-induced osteoporosis model (OVX) obtained tofacitinib BID by oral gavage for 6 weeks. For the serum-induced arthritis model (SIA) WT mice received tofacitinib or baricitinib BID by oral gavage for 14 days. Readout covered serum cytokine levels (ELISA), μ CT and mRNA analysis of bone (qPCR) and clinical scoring. Murine osteoclasts (OC) were assessed for differentiation (TRAP staining) and resorption (Von Kossa staining). Murine mesenchymal stem cell (MSC)-derived and primary osteoblasts (OB) were analyzed for differentiation (qPCR) and function (Alizarin red staining).

Results In unchallenged WT mice, treatment with tofacitinib increased trabecular bone density in tibia and reduced RANKL/OPG ratio in serum. These results, along with elevated trabeculae numbers, also applied to spinal bone in tofacitinib-treated OVX mice. In SIA, baricitinib and tofacitinib ameliorated disease manifestation and inhibited trabecular/cortical bone loss. *In vitro* JAK-inhibition exhibited no effect on differentiation and function of OCs. However, it enhanced OCN expression in MSC-derived OBs at day 1 after osteoblastic induction and led to decreased Igf1 and increased Dkk1 expression at day 7. Accordingly, primary OBs showed reduced RANKL expression during JAK-inhibition. Moreover, both MSC- and primary OBs responded to JAK-inhibition with increased mineralization.

Conclusions Our findings indicate that JAK-inhibition by tofacitinib and baricitinib increases bone formation *in vivo*, in both steady-state and pathological conditions, presumably as a result of increased mineralization capacity by osteoblasts.

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P149/006 HIGHER EXPRESSION OF SIGLEC-7/9 ON GRANULOCYTES AND MONOCYTES HAS A PROTECTIVE ROLE IN RHEUMATOID ARTHRITIS

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

Introduction Prominent features of rheumatoid arthritis (RA) are pain, joint swelling and erosion induced by massive infiltration of immune cells into the inflamed tissue. Among these immune cells, granulocytes and monocytes play a crucial role. Both express sialic acid-binding immunoglobulin-like lectins (Siglecs) –7 and –9 (in mice Siglec-E), trans-membrane proteins generally transmitting inhibitory signals through immunoreceptor tyrosine-based inhibitory motifs in their cytoplasmic tails. So far, there has been only limited research on the effects of Siglecs in RA.

Objectives To evaluate the potential role of Siglec 7- and –9 during disease progression in RA by comparing the expression on blood leukocytes with disease activity in RA patients. Additionally we monitored Siglec-E knockout mice in an experimental arthritis model.

Methods Blood samples from 45 healthy donors (HD) and 37 RA patients were analyzed by flow cytometry for Siglec-7 and –9 expressions on NK cells (CD14+/CD56+/CD3-), granulocytes (CD16+/CD19-) and monocytes (CD14+/CD16-). Expression was correlated with autoantibody positivity and disease severity.

Siglec-E knockout mice and wildtype controls were used in a serum transfer induced arthritis model and disease manifestation was monitored by measurement of paw swelling. Additionally we performed functional *ex vivo* assays to study Siglec impact on granulocytes and monocytes.

Results There was a diminished Siglec-7/9 expression on NK cells from RA patients, especially in highly CCP positive patients (>500 U/ml), while no differences were observed in neutrophils and monocytes compared to healthy controls. Interestingly we observed a correlation between a higher DAS28 score and lower Siglec-9 expression levels on granulocytes in seropositive patients.

Siglec-E knockout mice displayed higher disease scores and paw swelling in serum transfer induced arthritis, especially at the peak and the resolution phase. Functional studies unraveled that granulocytes are in a more active state if Siglec-7 and –9 are missing.

Conclusions Together our data support that Siglec-7/9 expression negatively correlates with pathogenesis and disease manifestation in RA patients. We showed *in vivo* and *ex vivo*, that Siglecs possess an important role concerning granulocyte and monocyte function and are capable of dampening immune response. Due to their inhibitory potential, Siglecs should be considered as potential therapeutic starting point in future studies.

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IMPACT OF RADON SPA ON PAIN AND THE IMMUNE SYSTEM OF PATIENTS WITH MUSCULOSKELETAL DISORDERS

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Introduction The pain reducing effects of certain natural springs containing the radioactive noble gas radon have been described for centuries. Today, one expects that small cumulative dose of radiation of about 0.3 mSv originating from radon progeny impacts on the immune system and bone metabolism of patients suffering from chronic painful degenerative and/or inflammatory diseases. However, osteoimmunological analyses of patients during radon spa were lacking.

Objectives We initiated the observational and explorative RAD-ON01 study to analyse for the first time the impact of radon spa on the immune status in the whole blood of 100 patients.

Methods Whole blood of the patients was drawn before and at weeks 6, 12 and 30 after therapy. Deep immunophenotyping was performed by multicolour flow cytometry and cytokine analyses by ELISA.

Results The RAD-ON01 study confirmed a long-lasting pain reduction. While the major immune cells were only marginally affected, in particular regulatory T cells and dendritic cells were temporarily increased and activation markers on immune cells were decreased. Further, a decrease of serum markers related to bone erosion was observed. The cytokine analyses showed that temporarily increased TGFβ following radon spa correlates with reduced pain perception of the patients. To exclude placebo effects, in November 2018 the RAD-ON02 study (EUDRACT: 2016-002085-31) started. With this prospective, temporarily placebo-controlled and double-blinded trial the evidence level of radon spa application and knowledge on osteoimmunological modes of action of radon should be improved.

Conclusions We conclude that patients with musculoskeletal disorders do benefit from radon spa and that osteoimmunological mechanisms are modulated by exposure of the patients to very low doses of radiation. Future randomized studies should improve the evidence level for radon as therapeutic option for chronic painful degenerative and/or inflammatory diseases.

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NOCICEPTIVE PAIN IN ACUTE EXPERIMENTAL SYNOVITIS IS PARTLY MEDIATED BY THE ALARMIN S100A9

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Introduction Inflammatory mediators like S100A8 and -A9 released by the synovium have been implicated in the regulation of pain. They may regulate pain either via direct stimulation of nerve endings in the synovium or via stimulation at the site of the dorsal root ganglia (DRG), enabling an increased phagocyte infiltration and causing sensitization.

Objectives To elucidate the role of S100A9 in the pain response after induction of an acute synovitis using streptococcal cell walls (SCW) as a trigger.

Methods Acute synovitis was induced by a single i.a. injection of SCW in the knee joint of C57Bl6 (WT) mice and S100A9^{-/-} mice, control mice received a saline injection. Serum S100A8/A9 levels were investigated by ELISA. Joint swelling and cell influx was assessed by ^{99m}Tc accumulation and histology. Pain response were investigated using an Incapacitance Tester (weight bearing), Catwalk (gait analysis) and von Frey's filaments (mechanical allodynia). Gene expression of inflammatory mediators and neuron activation markers in DRG were determined by q-PCR. Monocyte influx and protein expression was monitored by immunohistochemistry (IHC).

Results A single i.a. injection of SCW resulted in increased synovial and serum levels of S100A8 and S100A9 at day 1, which returned to basal levels at day 7. Joint swelling and cell influx were similar in WT and S100A9^{-/-} mice at day 1 day excluding a role for S100A8/9 in synovitis. WT mice showed a marked and significant decrease in percentage of weight bearing on the SCW injected hindpaw (28%) compared to saline injection (47%, p<0.001) at day 1, whereas S100A9^{-/-} mice did not. In addition, gait analysis showed increased 'limping' in the WT mice, whereas the S100A9^{-/-} mice did not. Both mouse strains showed a similar reduction of paw withdrawal threshold, excluding a role for S100A8/9 in allodynia. DRG showed no increased phagocyte infiltration after SCW injection and no change in gene expression of MCP-1, KC, IL-1β or TNF was measured. In addition, F4/80 staining was unchanged in both WT and S100A9^{-/-} mice. However, neuron activation markers NAV1.7, ATF3 and GAP43 were significantly increased at 1 day after SCW injection in WT mice, compared to saline injected mice (p=0.022, 0.004 and 0.030, respectively) and not in S100A9^{-/-} mice, which is in line in with the reduced pain response observed earlier in S100A9^{-/-} mice. The difference in NAV1.7 expression in the DRG was further confirmed at protein level with IHC.

Conclusions These findings show that S100A9 is an important mediator of inflammatory nociceptive pain response, and not of peripheral sensitization. During the acute phase of inflammation S100A8/A9 is likely involved via direct activation of nerve endings in the synovium and not via monocyte infiltration in the DRG.

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