metabolite ensuring linked choreography between fibroblast and macrophage movement in the synovium which may become uncoupled in disease. We propose that dysfunctional crosstalk between these two cell types due to high lactate levels, promotes inflammation and the establishment of persistent disease in RA. Targeting lactate/MCT's pathway may provide a novel therapeutic strategy, to restore cellular crosstalk and to reduce inflammation in RA patients.

REFERENCES:

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L-ARGININE REPROGRAMS OSTEOCLAST PURINE METABOLISM AMELIORATING BONE LOSS IN RHEUMATOID ARTHRITIS

S. Cao1,2, R. Song1,2, X. Meng1, K. Kacher1, M. Fuchs3, X. Meng1, Y. Li1, V. Taudte4, M. Kunz3, U. Schloetzer-Schrehardt5, U. Schleicher6, X. Chen2.

1Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Department of Internal Medicine 3-Rheumatology and Immunology Erlangen, Germany; 2Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Department of Rheumatology, Shanghai, China; 3Friedrich-Alexander University of Erlangen-Nürnberg, Chair of Medical Informatics, Erlangen, Germany; 4Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Experimental and Clinical Pharmacology and Toxicology, Erlangen, Germany; 5Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Department of Ophthalmology, Erlangen, Germany; 6Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Mikrobiologisches Institut – Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany

Background: Bone erosion is a clinical feature of rheumatoid arthritis related to disease severity and poor functional prognosis. Excessive osteoclast differentiation and insufficient osteoblast function are the main reasons for the erosive process in RA. Our previous investigation indicated that L-arginine supplementation not only diminished arthritic inflammation in the serum-induced arthritis (K/BxN) model but also deceased inflammatory joints osteoclast numbers (1).

Objectives: In the present study, we aim to investigate the metabolic action of L-arginine supplementation in RA, especially on periarticular bone erosion and systemic bone loss. We plan to depict the metabolic features of TNFα induced inflammatory osteoclasts after in vitro L-arginine supplementation.

Methods: Three murine arthritis models (serum-induced arthritis (K/BxN) model, collagen-induced arthritis model, and NTHFg mice model) were analysed in this study. L-arginine was supplemented within the drinking water after the arthritis model and gene parameters for osteoclast skeleton (spine) and periarticular skeleton (tibia) from the respective group were quantified by μCT. HE and TRAP staining were performed to address further the erosion area and osteoclast numbers in periarticular sites. In vitro osteoclast differentiation was conducted with or without L-arginine treatment, in the presence or not of TNFα activation. Seahorse and SCENITH analyses were adopted to delineate the metabolic features. JC-1 staining and transmission electron microscopy (TEM) were used to depict the mitochondria metabolism. RNA-Seq and mass spectrometry (MS) were performed to investigate the underlying molecular mechanism.

Results: Inflammation was diminished in all three arthritis models after L-arginine supplementation with a significant reduction in arthritic score. Moreover, an amelioration of periarticular bone erosion, systemic bone loss, and decreased osteoclast numbers in periarticular sites were observed in arthritic mice after L-arginine treatment. L-arginine also inhibited osteoclastogenesis in vitro. In particular under TNFα activation. Seahorse and SCENITH analyses indicated TNFα promoted glycolysis while blocking mitochondria-driven oxidative phosphorylations (OXPHOS) in pre-osteoclasts. Meanwhile, JC-1 staining and TEM images also showed that TNFα decreased mitochondria membrane potential and prompted damage of mitochondria. Surprisingly, L-arginine repressed the TNFα-induced OXPHOS while promoting ATP production. RNA-seq and MS data confirmed the boost of OXPHOS after L-arginine treatment under TNFα activation. To interfere with OXPHOS, L-arginine inhibited c-Jun thus altered arginase-1 and arginase-2 expression. Moreover, the increased ATP in L-arginine treated cells facilitated the metabolic shift from glycolysis to OXPHOS by increased mitochondrial respiratory capacity, con-tributing to the inhibition of osteoclastogenesis. Increasing Adenosine deaminase (ADA) is essential for the production of inosine and hypoxanthine due to the diminished inhibitory regulation of the transcription factor c-Jun.

Conclusion: These data strongly demonstrated that L-arginine ameliorates bone erosion in RA through metabolic reprogramming and perturbation of purine metabolism in osteoclasts. L-arginine might therefore benefit RA therapy by reducing joint inflammatory and also ameliorating bone destruction.

REFERENCES:
Polar plot of 3-way differential gene expression analysis on baseline synovial biopsies. Polar plot showing analysis of RNA-seq TPM counts tested for differential expression by DESeq2, comparing patients who failed tocilizumab and responded to rituximab (pro-RTX, blue if False Discovery Rate <0.05), who failed rituximab but responded to tocilizumab (pro-TOC, yellow if FDR<0.05), or who failed both drugs (refractory, red if FDR<0.05).

Genes in green show significant genes overlapping in pro-RTX and pro-TOC patients and genes in grey are statistically non-significant.

Conclusion: We provide novel insights into the cellular and molecular pathways underpinning multi-biologic resistance that define a refractory RA phenotype, characterised by a stromal/fibroblast signature.

REFERENCES:

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ALTERED IMMUNOLOGICAL CIRCADIAN RHYTHMS AND THE EFFECT OF TREATMENT WITH GLUCOCORTICOIDS ON CIRCADIAN RHYTHMS OF IMMUNE CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS: BRING BACK THE RHYTHM
S. Wilantri1,2, C. Strehi1,3, D. Abdarama1,2, T. Gaber1,2, R. Biesen1, F. Buttgereit1, on behalf of AG Buttgereit. 1Charité – Universitätsmedizin Berlin, Department of Rheumatology and Clinical Immunology, Berlin, Germany; 2Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), ein Institut der Leibniz-Gemeinschaft, Glucocorticoid and Bioenergetic, Berlin, Germany

Background: In rheumatoid arthritis (RA), pain, joint swelling, and stiffness follow a clear circadian pattern. Most of these symptoms are most pronounced in the early morning and primarily attributed to elevated levels of the key proinflammatory cytokines IL-6 and TNFα, which usually peak before clinical symptoms worsen (1). Synthetic glucocorticoids (GCs) are among the most prescribed drugs in the management of patients with RA. GCs have effects on almost every immune cell. GCs suppress expression of various cytokines, including IL-1β, TNFα, IL-6, and GM-CSF. Moreover, circadian rhythms of immune cells are known to be influenced by GCs. For example, GCs govern in part the rhythm of circulating CD4+ and CD8+ T cells.

Objectives: To identify circadian patterns for optimization of diagnosis and treatment strategies, we conducted a clinical study comparing healthy donors (HD) and patients with RA in terms of circadian rhythms. We examined the effect of treatment with GCs on circadian immune rhythms in patients with RA.

Methods: We recruited 12 HD and 13 patients with active RA (DAS28≥4.0) who either were (n=8) or, for comparison, were not (n=5) under current treatment with GCs on circadian immune rhythms in patients with RA.

RESULTS: Peripheral regulatory T cells are circadian in HD and RA, but the number was notably reduced in RA (Table 1). CD8+ T cells, CD14+ monocytes, and CD19+ B cells lost their circadian rhythms in RA, but these rhythms were restored with GC treatment. Circulating NK and NK T cells, which are not diurnal in HD, exhibited circadian fluctuations in RA. GC treatment suppressed diurnal pathological circulation rhythms of NK and NK T cells by reducing the amplitude by half. In monocytes, BMAL1, PER1, PER2, and REVERRA CR1 expression showed diurnal variation in RA, but not in HD. IL-6 exhibited a circadian pattern in both groups, and GC treatment showed no significant effects on IL-6. Serum IL-4, IL-5, and MIP3x showed circadian variation in HD only. The following cytokines were notably elevated in RA-patients: IFNγ, MIP1α, MIP1β, IL-1β, IL-2, IL-17A, and IL-21. GC reduced the expression of IL-10 significantly in RA.

Table 1. Circadian rhythms in the cellular, gene, and protein levels in HD, RA, and RA with ongoing GC treatment

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Circadian impact</th>
<th>Circulating immune cells</th>
<th>Clock genes in monocytes</th>
<th>Serum cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD vs. RA</td>
<td>Non-circadian in HD and RA</td>
<td>-</td>
<td>CLOCK, CRY2, DBP, RORA</td>
<td>Fractalkine, IFNγ, CXCL11, GM-CSF, MIP1α, MIP1β, IL-1β, IL-2, IL-4, IL-7, IL-8, IL-10, IL-13, IL-17A, IL-21, IL-23, IL-25</td>
</tr>
<tr>
<td>HD vs. RA</td>
<td>Circadian in HD and RA</td>
<td>CD3+</td>
<td>PER3</td>
<td>CD30, CD44, CD45, CD45RA, CD45RO, CD45RO/CD45R0, CD45R0, CD45RO/CD45RO</td>
</tr>
<tr>
<td>HD vs. RA</td>
<td>Circadian in HD and RA</td>
<td>CD8+ T cells, CD14+ monocytes, CD145+ B cells</td>
<td>BMAL1, PER1, PER2, REVERRA</td>
<td>IL-4, IL-5, MIP3x, IL-10, IL-12, IL-13, IL-17A, IL-21, IL-23</td>
</tr>
<tr>
<td>RA vs. GCs treated RA</td>
<td>Non-circadian in RA</td>
<td>CD8+ T cells, CD14+ monocytes</td>
<td>PER3, CYR1</td>
<td>CD8+ T cells, CD14+ monocytes, CD19+ B cells, CD19+ B cells</td>
</tr>
<tr>
<td>RA vs. GCs treated RA</td>
<td>Circadian in RA</td>
<td>NK cells, NK T cells</td>
<td>CYR1</td>
<td>CD8+ T cells, CD14+ monocytes, CD19+ B cells</td>
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</tbody>
</table>

Conclusion: With patients with RA, we found a certain loss of circadian rhythms and the establishment of ‘inflammatory’ rhythms. GC treatment in patients with RA resulted in three different types of effects on circadian rhythms at cartilage cell level: restoration, amplification, and attenuation. In conclusion, these findings provide new insights into the pathophysiology of circadian rhythms in RA that could be used to optimize diagnosis and treatment.

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Conclusion: In patients with RA, we found a certain loss of circadian rhythms and the establishment of ‘inflammatory’ rhythms. GC treatment in patients with RA resulted in three different types of effects on circadian rhythms at cartilage cell level: restoration, amplification, and attenuation. In conclusion, these findings provide new insights into the pathophysiology of circadian rhythms in RA that could be used to optimize diagnosis and treatment.

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