Low-Dose Radiotherapy Attenuates Experimental Autoimmune Arthritis by Inducing Lymphocyte Apoptosis

**Keywords:** Animal models, Adaptive immunity, Rheumatoid arthritis


**Background:** Disease-Modifying Anti-Rheumatic Drugs (DMARDs) have therapeutic effects on rheumatoid arthritis (RA) through the modulating immune responses of systemic and synovial. However, DMARDs may be used for an extended period, drug-related adverse effects can be a severe problem. Therefore, it is necessary to develop a therapeutic strategy to minimize the adverse effects of DMARDs, such as infection, abnormal liver function tests, and hematological abnormalities.

**Objectives:** Radiation therapy is one of the crucial therapeutic strategies for cancers. Low-Dose Radiotherapy (LDRt), the total dose may be given in fewer treatments or over a shorter period, has been tried to treat various inflammatory diseases, and its theoretical basis is poor. Therefore, this research aims to elucidate the mechanism and standardization of LDRt for RA.

**Methods:** The CIA (collagen-induced arthritis) and K/BxN mice used an experimental arthritic model representing RA. At the point of the highest arthritis symptoms, CIA and K/BxN mice were treated with LDRt. Then, the clinical score of the fore or hind paws was measured twice a week for 30 days. After 30 days of LDRt, the mice were sacrificed. Splenocytes, popliteal and inguinal lymph node cells were analyzed by flow cytometry to measure the level of activation, proliferation, inflammatory-cytokines, and apoptosis of immune cells. To evaluate whether LDRt is effective on human immune cells, RA patients' PBMC were treated with LDRt and analyzed by flow cytometry.

**Results:** LDRt did not affect the lymphocyte activation, proliferation, differentiation, and inflammatory cytokine secretion. However, LDRt enhanced lymphocyte apoptosis and decreased immune cell numbers and clinical scores in CIA and K/BxN mice. In addition, the degree of cartilage destruction was reduced by treating with LDRt in K/BxN mice. Likewise, the proportion of apoptotic lymphocytes was increased by LDRt in PBMC of a patient with RA, not fibroblast-like synoviocytes (FLS).

**Conclusion:** We uncovered that LDRt diminished experimental arthritis such as CIA and K/BxN via augmented lymphocyte apoptosis in draining lymph nodes. Thus, these findings suggest that LDRt can be a new therapeutic strategy with minimal adverse effects for RA.

**References:**


**Acknowledgments:** NIL.

**Disclosure of Interests:** None Declared.

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**AB0069**

**MEASUREMENT OF IL-33/ST2 AXIS IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS AND ITS ASSOCIATION WITH OTHER CYTOKINES IN SW982 AND U937 CELL LINES**

**Keywords:** Cytokines and chemokines, Rheumatoid arthritis, Cell biology

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**AB0070**

**PIM KINASES AS POSSIBLE THERAPEUTIC TARGETS IN INFLAMMATORY ARTHRITIDES**

**Keywords:** Biomarkers, Cell biology, Inflammatory arthritis


**Background:** Pim kinases are involved in the pathogenesis of RA and also indicates it to be a promising biomarker. Synovial fluid gives a natural microenvironment to cells in vitro studies showing increased expression of cytokines. This is the first study that shows a direct relation with IL-33 and LTb4 expression, thus proving the chemoattractive role of LTb4.

**References:**


Miya 2013:17137-17172.

**Disclosure of Interests:** None Declared.

**DOI:** 10.1136/annrheumdis-2023-eular.6239

**AB0068**

**LOW-DOSE RADIONERTHERAPY ATTENUATES EXPERIMENTAL AUTOIMMUNE ARTHRITIS BY INDUCING LYPHOCYTE APOPTOSIS**

**Keywords:** Animal models, Adaptive immunity, Rheumatoid arthritis


**Background:** An inflammatory maelstrom is a characteristic feature of autoimmune diseases including Rheumatoid Arthritis (RA). IL-33, a member of IL-1 cytokine family has been studied in animal models with induced RA and a few studies in RA patients. These studies have shown the exacerbation of IL-33, however little is known about its pathogenesis and association with other cytokines in vivo and in vitro.

**Objectives:** This study evaluated the expression of IL33 and ST2 in serum and Peripheral blood mononuclear cells (PBMC's) of active RA patients, in addition to other cytokines (IL-13, IL-5, IL-17) and biochemical parameters. TNF-alpha stimulated and synovial fluid (SF) conditioned growth medias were used to grow cell lines (SW982, U937) so as to have ambient physio-pathological microenvironment during experiments, typically of RA and then the levels of IL-33 was evaluated. Association of IL-33 with expression of LTb4 was also studied.

**Methods:** PBMC's and sera from 50 active RA patients and 50 healthy individuals were obtained. IL-33 and ST2 were measured using Real Time PCR with specific primers including internal controls. Sandwich ELISA was done for studying protein expressions. Cell lines were grown RPMI-1640 + 10% FBS for U937 and DMEM + Glutamine + 10% FBS for SW982 cells at 5% CO2.

**Results:** IL-33 and ST2 mRNA expression was significantly higher in patients with active RA, with estimated fold change of 3.533 (∆∆Ct = -1.82121) and 4.172 (∆∆Ct = -2.15229) respectively, as compared to controls. ELISA results revealed mean value of IL-33 to be significantly higher in RA patients than in controls (119.32±63.49 and 22.835±12.24 respectively). An increase in serum protein of ST2 was also observed in RA patients as compared to healthy controls (243.32±83.64 and 705.22±310.05). Out of the total (n=50), 35 RA patients showed a hike in serum and mRNA expression of IL-33 which also showed correlation with other disease biomarkers like anti-CCP CRP. DAS-28 CRP and HAO-DI. Significant increase in serum protein of IL-17 (1542.11±20.12) and IL-5 (624.21±78.09) were observed. SF stimulated SW982 cells showed a 1.8 fold increase in levels of IL-33 as compared to cells which were stimulated with TNF alpha alone. Further monocyte cell line was treated with recombinant human IL-33 antibody and showed direct association with levels of Leukotriene B4 (LTb4) expression.

**Conclusion:** Elevated IL-33 and ST2 in both serum and PBMC’s of RA patients showed correlation with the disease activity. Therefore, IL-33/ST2 signaling represents the involvement in the pathogenesis of RA and also indicates it to be a promising biomarker. Synovial fluid gives a natural microenvironment to cells in vitro studies showing increased expression of cytokines. This is the first study that shows a direct relation with IL-33 and LTb4 expression, thus proving the chemoattractive role of LTb4.

**References:**

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**Disclosure of Interests:** None Declared.

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lacking regarding the potential role of PIM kinases in spondyloarthropathy (SpA) such as psoriatic arthritis (PsA) and axial spondyloarthritis (AxSpA).

**Objectives:** To explore the potential role of PIM kinases in inflammatory arthritides, we evaluated PIM kinase expression at gene and protein level in patients with RA, PsA, and AxSpA compared to healthy controls (CTR).

**Methods:** PIMs expression was analysed in 26 (11 RA, 7 AxSpA, and 8 PsA) biologic DMARDs (bDMARDs)-naive arthritis patients and 13 controls. PIMs mRNA levels were determined in total RNA extracted from freshly isolated peripheral blood mononuclear cells (PBMC) by real-time semi-quantitative PCR. Protein levels were quantified in serum by ELISA test. Data were verified in Kruskal Wallis test followed by Dunn’s correction for multiple comparisons.

**Results:** All the samples showed expression of PIM1, 2, and 3 kinases both at gene and protein level, with PIM 3 being the less expressed protein. A trend towards lower expression of PIM1 was appreciated in AxSpA samples compared to other groups, reaching statistical significance only for serum protein levels compared to controls (p < 0.05). No significant differences were found in PIM2 and PIM3 expression, although all arthritis patients showed a trend towards higher serum levels compared to controls, not mirrored at gene expression levels.

**Conclusion:** PIM 1 mRNA expression in PBMCs seems to mirror PIM1 protein expression in the serum in all groups, supporting this molecule as a candidate biomarker. Controls, RA and PsA patients displayed similar levels, whereas AxSpA patients showed a trend towards reduced PIM1 levels deserving further investigation. Conversely, serum PIM2 and PIM3 levels did not mirror PIM2 and PIM3 gene expression in resting PBMC. Unlike PIM1, the expression of PIM2 and PIM3 have never been studied in PsA and AS, and additional data are required to explore the potential relevance of PIM2 and PIM3 in SpA pathophysiology. Further studies are also required to evaluate PIM kinases modulation after in vitro cell stimulation, the differential expression in PBMC subpopulations, and the impact of disease activity and concurrent disease-modifying medications.

**REFERENCES:**


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**Table 1.** PIM1, 2, and 3 expression in rheumatoid arthritis, axial spondyloarthritis, psoriatic arthritis and healthy controls. Data are expressed as medians with interquartile ranges [25th–75th percentiles].

<table>
<thead>
<tr>
<th>PIM 1</th>
<th>RA</th>
<th>AxSpA</th>
<th>PsA</th>
<th>CTR</th>
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<tbody>
<tr>
<td>Gene expression in PBMC (in respect to GAPDH)</td>
<td>0.17</td>
<td>0.12</td>
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<td>Serum protein concentration (ng/ml)</td>
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<td>Gene expression in PBMC (in respect to GAPDH)</td>
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<td>Serum protein concentration (ng/ml)</td>
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<th>RA</th>
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<th>PsA</th>
<th>CTR</th>
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<td>Gene expression in PBMC (in respect to GAPDH)</td>
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<td>Serum protein concentration (ng/ml)</td>
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Disclosure of Interests: None Declared.

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**AB0071**

**PRE-CLINICAL EVALUATION OF ANTI-ANGIOGENIC EFFECTS OF CANDIDATE AGENTS FOR MOLECULAR DIAGNOSTIC IMAGING AND THERAPY IN RHEUMATOID ARTHRITIS**

**Keywords:** Rheumatoid arthritis, Synoviom, Imaging

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**Background:** The role of pathological angiogenesis is widely recognized in cancer and various inflammatory diseases, including rheumatoid arthritis (RA), in RA, angiogenesis and inflammation are mutually dependent and play a cooperative role in the disease progression. Angiogenesis mainly relies on the interaction of vascular endothelial cells (ECs), fibroblasts, macrophages, and the extracellular matrix. This phenomenon also contributes to the infiltration of inflammatory cells into the joints, resulting in more synovial inflammation and structural damage. In this process, a number of soluble and cell surface-bound angiogenic mediators are involved providing candidate targets for diagnosis and therapy [1, 2]. Anti-angiogenesis strategy has been widely used in tumor treatment and its potential value in RA as a single or in combination therapy need to be further explored.

**Objectives:** To assess potential anti-angiogenic effects of selected compounds undergoing different mechanisms of action in preclinical models of angiogenesis for exploitation as candidate agents for molecular imaging and/or therapeutics purposes.

**Methods:** Two in vitro models of angiogenesis were employed in this study: (i) a scratch assay assessing the closure rate of scratches made in human microvascular endothelial cells (HMEC-1) [3] in the presence of drugs for 24 hr, and (ii) a 3D-spheroid model of angiogenesis composed of ECs and fibroblasts, assessing the impact of drugs on EC sprout formation after 40 hr of incubation. Sprott quantification was performed via confocal microscopy and digital image analysis [2]. Candidate anti-angiogenic compounds examined in this study included: sunitinib (pan-kinase inhibitor), a small molecule NF-κB-Inducing kinase inhibitor (NIK), tofacitinib (selective JAK1/3 inhibitor) and fluciclatide (RGD-peptide targeting α5β1 and αvβ3 integrins) and tested in concentration range of 0.1 μM. An MTT-proliferation inhibition assay was used to monitor any drug-induced cytotoxicity.

**Results:** In scratch assays, sunitinib exhibited irreversible inhibition (max 75% at 10 μM after 24 hr incubation) compared to NIKi which had a transient inhibitory effect (max 59% at 10 μM after 8 hr incubation). No apparent effects were observed for tofacitinib up to concentrations of 10 μM. Interestingly, fluciclatide, induced morphological changes at concentration of 0.1 μM, impairing scratch closures. Next, the anti-angiogenic effects of the compounds were verified in the 3D-spheroid model that more closely mimics the in vivo situation. Here, marked blockade of sprott formation was noted at 0.1 μM for sunitinib, and 1.25 μM for NIKi, tofacitinib and fluciclatide. MTT assays indicated that none of the observed drug-effects were due to induction of cytotoxicity at the indicated concentrations.

**Conclusion:** This study identified various compounds (sunitinib, NIKi, tofacitinib and fluciclatide) exhibiting potential anti-angiogenic properties in vitro, encouraging their further evaluation as radiouclide imaging and therapeutic agents for diagnosis and therapeutics in vivo.

**REFERENCES:**


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