Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic proliferation of synovial cells and destructive polyarthritis. Various kinds of cells are involved in the pathogenesis of RA, including myeloid cells, lymphocytes, and synovial fibroblasts. It has been reported that there are abnormal myeloid cells in bone marrow of RA patients (Ochi, et al., Arthritis Res. Ther., 2007). In addition, myeloid cells differentiate into monocytes/macrophages, which can thereafter differentiate into osteoclasts that mediate bone destruction in RA. In fact, we have previously reported CD14+ monocytes differentiated from induced pluripotent stem cells (iPSCs) derived from RA patients expressed more mRNA of receptor activator of nuclear factor-kappa B (RANK) than those from a healthy donor, non-onset family member (NOF), resulting in the accelerated osteoclastogenesis (Ito, et al., The 36th annual meeting of the Japanese society of inflammation and regeneration, 2015). However, the question remains if distinct monocyte differentiation besides high RANK expression may also be involved in the accelerated osteoclastogenesis in RA.

Objectives: To investigate whether there is distinct monocyte differentiation between a RA patient and NOF.

Methods: iPSCs have been established from skin fibroblasts from a RA patient. For the controls, NOF of the patient are recruited as a control in order to adjust hereditary background as much as possible. Monocytes were induced from each iPSC clone culture on feeder-free conditions using a previously reported method (Cui, et al., Front. Cell Dev. Biol., 2021). Floating cells were sequentially collected on days 10, 14, 18 and 22 during induction of monocytes from iPSCs. Surface phenotypes and the expression of differentiation markers of monocytes were analyzed with flow cytometry using the corresponding antibodies.

Results: RA-iPSCs differentiated into CD43+ (hematopoietic marker) cells earlier than NOF-iPSCs did (the data shown in Table 1). In addition, the proportion of CD45+ in CD43+ cells (myeloid progenitors) was achieved in 93.0±4.8% (RA) and 51.0±23.1% (NOF) on day 18 (p=0.029) (the data shown in Table 1). The proportion of CD11b+CD45+ cells (monocytes and granulocytes) derived from RA-iPSCs was not significantly different from that from NOF-iPSCs. However, the proportion of CD45+ in CD11b+CD45+ cells (monocytes) derived from RA-iPSCs was significantly higher than that from NOF-iPSCs on day 18 and 22 (the data shown in Table 1). CD45+ in CD11b+CD45+ cells were mostly CD14+ cells. The proportion of CD115+, macrophage colony-stimulating factor receptor (MCSF-R), in CD14+ cells induced from RA-iPSCs tended to increase compared with NOF-iPSCs as the days of differentiation progressed (the data shown in Table 1).

Conclusion: Hematopoietic stem cells differentiate into myeloid progenitors in monocyte differentiation in RA earlier than those in NOF. Moreover, they differentiate into more CD14+CD11b+CD45+ monocytes which are likely to express more MCSF-R as compared with those in NOF. We speculate that these distinct monocyte differentiation may play an accelerated osteoclastogenesis in RA.

Table 1. Proportion of cells differentiated from RA-iPSCs and NOF-iPSCs

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Proportion of cells differentiated from RA-iPSCs and NOF-iPSCs</th>
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<tbody>
<tr>
<td></td>
<td>CD43+ cells</td>
</tr>
<tr>
<td></td>
<td>RA/iPSCs</td>
</tr>
<tr>
<td></td>
<td>(%) ± SD</td>
</tr>
<tr>
<td>10</td>
<td>52.0±6.5</td>
</tr>
<tr>
<td>14</td>
<td>49.8±8.1</td>
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<tr>
<td>18</td>
<td>44.4±11.0</td>
</tr>
<tr>
<td>22</td>
<td>32.3±4.1</td>
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</tbody>
</table>

D = Day, * = significant
AB0068

LOW-DOSE RADIOTHERAPY ATTENUATES EXPERIMENTAL AUTOIMMUNE ARTHRITIS BY INDUCING LYMPHOCYTE APOPTOSIS

Keywords: Animal models, Adaptive immunity, Rheumatoid arthritis

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Background: Disease-Modifying Anti-Rheumatic Drugs (DMARDs) have therapeutic effects on rheumatoid arthritis (RA) through the modulating immune responses of systemic and synovial tissues. However, DMARDs are used for an extended period, drug-related adverse effects can be a severe problem. Therefore, it is necessary to develop a treatment 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. Low-Dose Radiotherapy (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/Bx mice. In addition, the degree of cartilage destruction was reduced by treating with LDRT in K/Bx 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/Bx mice via augmented lymphocyte apoptosis in draining lymph nodes. Thus, these findings suggest that LDRT can be a new therapeutic strategy with mild adverse effects for RA.

REFERENCES:

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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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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 were 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(ΔΔCt = -1.82121) and 4.172 (ΔΔCt = -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 (2434.328±834.60 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;112±120.21) 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 monocyctic 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 involved in the pathogenesis of RA and also indicates it to be a promising biomarker. Synovial fluid gives a natural microenvironment to cells in 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 chemotactic role of LTB4.

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Disclosure of Interests: None Declared.

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AB0070

PIM KINASES AS POSSIBLE THERAPEUTIC TARGETS IN INFLAMMATORY ARTHRITIDES

Keywords: Biomarkers, Cell biology, Inflammatory arthritis

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Background: PIM kinases are involved in T-cell amplification and survival, proliferation and apoptosis. These proteins have been extensively studied in tumorigenesis, whereas their role in autoimmune and chronic inflammatory diseases remain unclear. PIM kinases are involved in T-cell amplification and differentiation in inflammatory Th1 and Th17 effector T cells subsets. Recent studies suggest that the expression of PIM1 is upregulated in fibroblast-like synoviocytes and CD4+ T cells from patients with rheumatoid arthritis (RA). However, data are