AB0066  ACCELERATED MONOCYTE DIFFERENTIATION AND HIGH EXPRESSION OF MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR IN THE MONOCYTES MAY BE INVOLVED IN ACCELERATED OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic proliferation of synovial cells and destructive polyarthritides. 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 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 factorkappa 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 these controls, NOF of the patient are recruited as a donor 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 81.0±2.13% (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 (the data shown in Table 1). CD45+ in CD11b+CD45+ cells were mostly CD14+ cells. The proportion of CD11b+ macrophage colony-stimulating factor receptor (M-CSFR), 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 monocytic differentiation in RA earlier than those in NOF. Moreover, they differentiate into more CD14+CD11b+CD45+ monocytes which are likely to express more M-CSFR 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

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<tr>
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<th>RA-iPSCs</th>
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<th>RA-iPSCs</th>
<th>NOF-iPSCs</th>
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<tr>
<td></td>
<td>(% ± SD)</td>
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<td>10</td>
<td>52.0 ± 6.5</td>
<td>23.4 ± 2.8</td>
<td>0.002</td>
<td>36.3 ± 18.8</td>
<td>35.4 ± 14.5</td>
<td>0.953</td>
<td>27.3 ± 3.2</td>
<td>30.9 ± 9.8</td>
<td>0.584</td>
<td>28.0 ± 4.1</td>
<td>30.9 ± 8.9</td>
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<td>14</td>
<td>49.8 ± 8.1</td>
<td>43.7 ± 20.8</td>
<td>0.662</td>
<td>78.4 ± 11.4</td>
<td>59.2 ± 25.5</td>
<td>0.298</td>
<td>36.8 ± 14.3</td>
<td>30.8 ± 8.9</td>
<td>0.769</td>
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<td>44.4 ± 11.0</td>
<td>47.7 ± 10.9</td>
<td>0.735</td>
<td>93.0 ± 4.8</td>
<td>51.0 ± 21.3</td>
<td>0.029</td>
<td>52.9 ± 7.2</td>
<td>28.6 ± 2.6</td>
<td>0.005</td>
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<td>22</td>
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<td>47.6 ± 12.6</td>
<td>0.116</td>
<td>87.0 ± 18.6</td>
<td>80.7 ± 6.6</td>
<td>0.611</td>
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<td>0.001</td>
<td>82.7 ± 7.5</td>
<td>45.7 ± 22.1</td>
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</table>

D = Day, * = significant

REFERENCES: NIL.

Disclosure of Interests: NIL.

AB0067  MODE OF ACTION AND TARGET INVESTIGATION OF THE NEW ANTI-RHEUMATIC DRUG RABEXIMOD.

Keywords: Innate immunity, Rheumatoid arthritis, Cell biology

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Disclosure of Interests: None Declared.

Acknowledgements: NIL.

REFERENCES: NIL.

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 known to be 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, but 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 lymphoid tissues from the fore or hind paws were measured twice a week for 30 days. After 30 days of LDRT, the mice were sacrificed. Splenocytes, popliteal and inguinal lymphoid 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' PBMCs 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, the mice were sacrificed. Splenocytes, popliteal and inguinal lymphoid 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' PBMCs were treated with LDRT and analyzed by flow cytometry.

Conclusion: We uncovered that LDRT diminished experimental arthritis such as CIA and K/BxN mice 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:

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Disclosure of Interests: None Declared. DOI: 10.1136/annrheumdis-2023-eular.6239