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 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 (Ouchi, 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 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 51.0±21.3% (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 CD45R+ 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). CD45R+ 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 stages 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+CD45R+ monocytes which are likely to express more M-CSFR as compared with those in NOF. We speculate that these distinct monocyte differentiation may play in accelerated osteoclastogenesis in RA.

REFERENCES: NIL.

Acknowledgements: NIL.

Disclose of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.5310

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

Background: Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease which primarily affects the joints [1]. Numerous agents are currently used to modify the natural course of the disease, however, several RA patients still do not reach clinical remission [2]. A recently developed quinolone analogue named Rabeximod (RBM) showed clinical benefits comparable to TNF-α blockers in a mouse model of collagen antibody-induced arthritis [3]. Moreover, in a phase IIa clinical trial a significantly higher clinical efficacy of RBM than Methotrexate versus Methotrexate alone has also been confirmed.

Objectives: Since RBM’s exact biologic target has not been entirely established yet, our objective is to investigate its mode of action at the cellular and molecular level.

Methods: Two in vitro models were developed: i) Toll-like receptor (TLR)-4 and TLR-2-stimulated peripheral blood mononuclear cells (PBMCs) cultured for 24-hours and ii) Monocytes-derived macrophages (MDMs), cultured for 96-hours in persisting inflammation conditions mimicking the different phases of ACPA-positive rheumatoid synovial inflammation: recruitment, initiation, development, and persistence of the inflammatory process. PBMCs-transcriptomic analysis was performed using a NanoString nCounter SPRING Profiler (customised panel). Cytokine production was measured by ELISA.

Results: RBM induced dose-dependent cell mortality. Among PBMCs, monocyte viability was preferentially affected compared to lymphocytes. RBM treatment reduced IL-6 and TNF-α production both in TLR4/TLR2-stimulated PBMCs and MDMs supernatants at 24h (PBMCs) and 10-, 24-, 48- and 72-hour time-points (MDMs). RBM-induced down-regulation of IL-6 and TNF-α in PBMCs was also obtained when PBMCs were pre-incubated for 7 hours prior to TLR activation (without additional RBM), suggesting an internalisation process. After 24h, RBM induced the downregulation of several pro-inflammatory genes in TLR-4 and-2 stimulated PBMCs, including IL-6, TNF-α, CSF1, FCN1, and SPP1. Finally, after 24-hours, MDMs treated with RBM developed an altered morphology independently of the frequency of treatment exposure and the stage of inflammation.

Conclusion: Overall, our data suggest that RBM acts predominantly on monocytes/macrophages and can inhibit the production of pro-inflammatory molecules playing a crucial role in RA.

REFERENCES:

Acknowledgements: NIL.

Disclosure of Interests: Giulia Maria Ghirardi: None declared, Alessandra Nerviani: None declared, Liliane Fossati-Jimack: None declared, Elena Pontarini: None declared, Paola Italiani: None declared, Federico Pratesi: None declared,

G. M. Ghirardi1, A. Nerviani1, L. Fossati-Jimack2, E. Pontarini1, P. Italiani2, F. Pratesi3, S. Abdelmoaty4, C. Högerkorp4, C. Pitzalis1. 1William Harvey Research Institute, Experimental Medicine & Rheumatology, London, United Kingdom; 2Institute of Biochemistry and Cell Biology, Centro Nazionale delle Ricerche, CNR, Napoli, Italy; 3University of Pisa, Department of Clinical and Experimental Medicine, Pisa, Italy; 4Cyxone, AB, Cyanine, AB, Malmö, Sweden

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

<table>
<thead>
<tr>
<th></th>
<th>RA/iPSCs</th>
<th>NOF/iPSCs</th>
<th>p value</th>
<th>RA/iPSCs</th>
<th>NOF/iPSCs</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD43+</td>
<td>(% ± SD)</td>
<td>(% ± SD)</td>
<td></td>
<td>(% ± SD)</td>
<td>(% ± SD)</td>
<td></td>
</tr>
<tr>
<td>CD45+</td>
<td>CD43+ cells</td>
<td>CD45+ cells</td>
<td></td>
<td>CD45+</td>
<td>CD45+ cells</td>
<td>CD45+ cells</td>
</tr>
<tr>
<td>D</td>
<td>CD45+ in CD43+ cells</td>
<td>CD45+ in CD43+ cells</td>
<td></td>
<td>CD45+ in CD43+ cells</td>
<td>CD45+ in CD43+ cells</td>
<td></td>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<td>18</td>
<td>44.4±11.0</td>
<td>47.2±10.9</td>
<td>0.735</td>
<td>93.0±4.8</td>
<td>51.0±21.3</td>
<td>0.029</td>
</tr>
<tr>
<td>22</td>
<td>32.3±4.1</td>
<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>
</tr>
</tbody>
</table>

D = Day, * = significant

REFERENCES: NIL.

Disclose of Interests: Miho Murakami: None declared, Norhiro Nishimoto: None declared, Akira Chugai Pharmaceutical Co. and Eisi Co., Ltd.:

DOI: 10.1136/annrheumdis-2023-eular.5310

Scientific Abstracts

Downloaded from http://ard.bmj.com/ on July 21, 2023 by guest. Protected by copyright.