FRI0060 MONOCYTE-DERIVED MICROPARTICLES IN CIRCULATION OF PATIENTS WITH RHEUMATOID ARTHRITIS: A NOVEL BIOMARKER FOR THE DISEASE ACTIVITY

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Background: Rheumatoid arthritis (RA) is a systemic connective tissue disease and characterized by inflammation of synovium of multiple joints, however, its pathogenesis is still unclear (1). Physiologically, microparticle (MP) is categorized as a membrane microvesicle which contains various humoral factors such as cytokines or growth factors and 0.1–1 µm in size (2). And MPs can distribute systemically through circulation. MP is released from almost all cell types and induced by stimulation, stress or apoptosis (3). Thus, we hypothesized that MPs derived from various cells can distribute to the lesion of RA and be associated with systemic disease process of RA.

Objectives: To clarify the source of MPs in circulation of RA and elucidate their role in the pathogenesis and the possibility for application as a novel disease marker.

Methods: Twenty patients with RA and 13 healthy controls were involved. Using gradient centrifugation, platelet-rich plasma was isolated from whole blood and applied to flow cytometry. MP is defined as a vesicle less than 1 µm in diameter and Megamix[®] was used to set a gating condition to detect microparticles (4). Sources of MP (MP subsets) were identified by the expression of cell-specific markers using flow cytometry with fluorescence-conjugated antibodies as follows; platelets MP: CD41⁺CD31⁺ MP, endothelial cell MP: CD41⁺CD31⁺ MP, immune cell MP: CD45⁺CD41⁺ MP, MP derived other cells: CD41⁺CD31⁺CD45⁺ MP. Correlation between clinical information and proportion of MP subsets was also analyzed. Comparisons between two groups were examined by Mann-Whitney U-test with Bonferroni correction. Significant difference was defined as corrected *p* value (*P*_c) <0.05.

Results: Mean age of 20 patients with RA was 56±14 years, 95% was female, disease duration was 8.5±8.0 years, and DAS28-ESR was 2.0±1.1. Methotrexate was administered to 75% and average dose was 8.0±2.4 mg/week. Platelet-derived MP covered the largest proportion in all groups. Interestingly, proportion of immune-cell-derived MP was higher (*P*_c<0.006) in patients with RA compared to controls. Proportion of immune-cell-derived MP was positively correlated with inflammatory markers such as CRP and composite disease markers such as DAS28-ESR. Furthermore, when stratified by monocyte fraction and non-monocyte fraction based on CD14 expression, proportion of monocyte-derived MP showed higher (*P*_c<0.01) in patients with RA compared to controls, but non-monocytes MP did not, and that of monocyte-derived MPs was also correlated with disease markers for activity of RA.

Conclusions: Proportion of circulating immune-cell-derived MPs, especially monocyte-derived MPs, increased in RA and correlate with disease activity. These results suggest that monocyte-derived MPs can be measured as a novel biomarker and involved in the disease process of RA.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4599

FRI0061 IDENTIFICATION OF CITRULLINATION SITES SPECIFIC FOR PEPTIDYLARGININE DEIMINASE 2 (PAD2) AND PAD4 IN FIBRINOGEN FROM SYNOVIAL FLUID OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Protein citrullination, i.e. conversion of arginine residues into citrulline residues, is a post-translational modification catalyzed by PAD, and is an important pathophysiological determinant in conditions such as Rheumatoid arthritis (RA). Identification of citrullination sites on putative autoantigens is likely to enhance our understanding of PAD's substrate specificity.

Objectives: Citrullinated fibrinogen is an autoantigen linked to the pathophysiology of RA. We have applied a novel MS-based proteomics approach to estimate the degree of citrullination in synovial fluid (SF) from RA patients, and compare to fibrinogen citrullinated *in vitro* by PAD2/4, the most important PAD isoforms involved in RA. The estimated degree of citrullination induced by the two isoforms is also compared to evaluate their relative impact.

Methods: Fibrinogen was citrullinated *in vitro* by PAD2/4 and citrullination sites were identified by LC-MS/MS on a Q-exactive orbitrap following proteolytic digestion with Lys-C. These *in vitro* citrullination profiles were compared to

those observed in SF fibrinogen of four RA patients with varying DAS28 scores, CRP levels and leukocyte counts. DAS28 scores >5.1 and ≤2.4 correspond to moderate to severe and low activity of disease respectively. Patients with high inflammatory activity gave high CRP level and leukocyte count values.

Results: A total of 52 citrullination sites were identified. Overall, PAD2 generated higher number of identified sites and higher degree of citrulline occupancy at given sites than PAD4. In fibrinogen from SF, 38 citrullination sites were identified, of which 23 have not been previously reported. Several of these sites were identified in more than one patient, and were regarded as hotspots. Fibrinogen from patients with high DAS28 levels contained markedly more citrullination sites and higher citrulline occupancy.

Conclusions: Study suggests that PAD2 citrullinates fibrinogen more efficiently than PAD4 and citrullination of certain sites in fibrinogen from SF reflects disease activity. Identification of such sites may have diagnostic or prognostic value in RA and other inflammatory disorders.

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Acknowledgements: The Danish Council for Independent Research Natural Sciences (grant number 11–106246) and the Velux foundation. The Danish Research Foundation, Nordic Bioscience A/S and DTU are acknowledged for a joint PhD scholarship. LS was supported by the Ministry of Health of the Czech Republic (grant #00023728).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5950

FRI0062 SYNOVIAL FLUID TREG CELLS SECRETE IL-17 AND AT THE SAME TIME ARE POTENT SUPPRESSORS OF TRESP CELL PROLIFERATION, TNF ALPHA AND IFN GAMMA PRODUCTION

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Background: IL-17-expressing FoxP3 regulatory T cells have been described, and their suppressive capacity has been questioned. An inflammatory environment seems to favor IL-17 secretion by regulatory CD4⁺CD25⁺FoxP3⁺ T cells.

Objectives: To assess the suppressive function and IL-17 producing capacity of CD4⁺CD25⁺CD127⁺FoxP3⁺ T cells in the synovial fluid of RA patients (RASFD).

Methods: Synovial fluid was drawn from 35 patients with established RA who were receiving methotrexate and low-dose oral prednisone. The frequency of CD4⁺CD25⁺CD127⁺FoxP3⁺ T cells was assessed by flow cytometry. Total CD4⁺ T cells, CD4⁺CD25⁺CD127⁺ T reg cells and CD4⁺CD25⁺ Tresp cells were isolated by Ficoll-Hypaque gradient, followed by sorting. After isolation, cells were stimulated for 5 hours with PMA/ionomycin or cultured for 5 days in flat-bottom 96-well plates coated with an anti-CD3 monoclonal antibody. Treg cell function was assessed using two different approaches: A. The regulatory function of natural proportions of Tregs was inferred by comparing the proliferative and cytokine responses of total CD4⁺ T cells (TCD4T) versus CD25⁺ depleted CD4⁺ T cells (CD4⁺CD25⁺ T cells or Tresp cells); B. The per cell suppressor potency of Tregs was assessed in cocultures of isolated Tregs with Tresp, established at different Treg/Tresp ratios. Proliferation was determined by 3Hthymidine incorporation and CFSE dilution; cytokine secretion was measured by ELISA of culture supernatants.

Results: A high proportion of CD4⁺CD25⁺CD127⁺ T cells was present in RASFD (mean ± SD, 21.1%±6.2), which is significantly higher than reported frequencies of this cell population in the peripheral blood of both RA and healthy subjects. These RASFD CD4⁺CD25⁺CD127⁺ T cells expressed FoxP3 but did not express CD69. The proliferation rate, TNFα and IFNγ secretion were significantly higher for isolated Tresp as compared with TCD4T cells, indicating that natural proportions of Treg cells present in the synovial fluid of RA are functionally suppressive. Surprisingly, TCD4T cells secreted higher amounts of IL-17 as compared with Tresp cells, although the difference did not reach statistical significance. On a per cell basis, RAPB Tregs were potent suppressors of Tresp proliferation, TNFα and IFNγ but not of IL-17 secretion; in fact IL-17 secretion did not decrease but was enhanced in the presence of increasing proportions of Treg cells. Isolated Treg cells did not proliferate or produce cytokines when cultured alone in anti-CD3 coated plates. However, in the presence of plate-bound anti-CD3 plus anti-CD28 and recombinant human IL-2, isolated Treg cells secreted significant amounts of IL-17 whereas no TNFα or IFNγ could be detected in supernatants.

Conclusions: CD4⁺CD27⁺CD127⁺ FoxP3⁺ Treg cells present in the synovial fluid of RA patients are potent suppressors of Tresp proliferation, TNFα and IFNγ secretion, and at the same time produce significant amounts of IL-17.