Results: Neutrophils from healthy donors exhibit enhanced NET formation when incubated with or from SLE patients’ plasma (P<0.001), compared to HC plasma. Their NETs are decorated with ox-mtDNA (P<0.01). SLE patients’ mtDNA elicits increased NETs (P<0.001) decorated with ox-mtDNA (P<0.001), which are both not entirely abolished after DNase I treatment. Formation of NETs and ox-mtDNA extrusion seems to be partially regulated through the type I IFN pathway; JAK inhibition with tocilizumab diminishes, however not entirely, NET formation and ox-mtDNA release (56% and 60% decrease, respectively). mtDNA amounts correlate with B-CD45 (R²=0.87; P<0.0001) and type I IFN levels (R²=0.60; P=0.001) in SLE patients’ plasma. apL Ab-positive SLE patients were characterized by higher plasma levels of mtDNA (P=0.004), but not nDNA, compared to aPL Ab-negative SLE patients. Interestingly, aPL Ab-positive and aCL Ab-positive SLE patients’ plasma demonstrated a higher pro-NEtotic capacity.

Conclusion: Oxidative damage of mtDNA promotes the formation of NETs, is particularly interferogenic, and NETs relate to the antiphospholipid antibody positivity in SLE patients’ circulation. Circulatory ox-mtDNA might promote endothelial damage and vasculitis in SLE. A significant link between aPL, namely aCL IgG, and circulating mtDNA in SLE patients is evident, potentially aggravating the inflammatory state linked to disease severity and promoting thrombotic events.

REFERENCES:

Disclosure of Interests: UW is co-inventor of patents owned by Freiburg University; NY is co-inventor of patents owned by Freiburg University.

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**AB0027**

ANTI-INFLAMMATORY EFFECTS OF TNF INHIBITOR AND METHOTREXATE ON IMMUNE CELLS FROM PERIPHERY AND SYNOVIAL FLUID: EX-VIVO STUDY

Keywords: Disease-modifying drugs (DMARDs), bDMARD

S. Gertz1, V. Furer1, A. Polachek1, O. Elkayam1. 1From the Department of Rheumatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; Affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Rheumatology, Tel Aviv-Yafo, Israel

Background: Methotrexate (MTX) and TNF inhibitors (TNFi) are pivotal treatments for inflammatory arthropathies. Even to date, the mode of action of these drugs on diverse inflammatory cell populations is not well characterized.

Objectives: To study the impact of MTX and TNFi on the proliferation of peripheral blood mononuclear cells (PBMCs) and the intermediate monocytes (CD14+CD16+) frequency in synovial fluid mononuclear cells (SFMCs). The study focused on monocytes expressing the CD16+ as these cells promote synovial inflammation producing pro-inflammatory cytokines, including TNF, IL-1β, and IL-6.

Methods: In this pilot exploratory study, we analyzed ex-vivo healthy donors PBMCs (n=19) proliferation with or without phytohemagglutinin (PHA) and PHA in presence of MTX and/or TNFi. Next, the effect of these drugs on %CD14+CD16+ cells derived from psoriatic arthritis (PsA) patients SFMCs (n=11) was determined. Both assays were analysed by flow cytometry.

Results: Healthy donors PBMCs proliferation was inhibited by MTX (0.01 µg/ml and 0.1 µg/ml; 41.9±2.3 and 86.3±1.8, respectively) and to a lower extent by TNFi (infliximab, IFX) (1 µg/ml and 10 µg/ml; 43.1±2.5 and 49.4±2.5, respectively), whereas the combination of IFX 10 µg/ml and MTX 0.1 µg/ml led to the highest inhibition (91.0±2.0) (Figure 1A and B). In PsA patients derived SFMCs, IFX 10 µg/ml significantly reduced (p<0.01) the %CD14+CD16+ cells (5.5±1.3) as compared to medium control (10.9±2.4), whereas MTX 0.1 µg/ml had only modest effect on this cell population (10.2±1.4). The combination of IFX 10 µg/ml and MTX 0.1 µg/ml resulted in the greatest reduction of %CD14+CD16+ cells (5.1±1.1) (Figure 1C and D).

SFMCS from PsA patients (n=11) were co-cultured for 7 days with the drugs or with medium alone. (C) Representative plots showing CFSE-labeled healthy subject PBMCs. In the upper panel: Proliferation was measured either without PHA (no proliferation) or with PHA alone (maximal proliferation). Proliferation extent is shown in the left side of each plot. Lower panel: PHA with different drugs. Each image indicates %proliferation inhibition exerted by each drug. (B) Graph shows mean %proliferation inhibition, *p<0.05, **p<0.01.

Disclosure of Interests: None Declared.

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**AB0028**

LEUCOCYTE ABNORMALITIES IN SYNOVIAL FLUID OF DEGENERATIVE AND INFLAMMATORY ARTHROPATHIES

Keywords: Arthritis, inflammatory, synovial fluid, cytokines

C. Baggetto1, R. Luisetto2, C. Boscaro3, A. Scanu4, R. Ramonda1, M. Albiero5, P. Stumbo5, F. Oliviero1. 1University of Padova, Rheumatology Unit - Department of Medicine, Padova, Italy; 2University of Padova, Department of Surgery, Oncology and Gastroenterology-DISCOG, Padova, Italy; 3University of Padova, Department of Medicine; Veneto Institute of Molecular Medicine, Padova, Italy; 4University of Padova, Department of Woman’s and Child’s Health, Padova, Italy

The immunomodulatory effects induced by TNFi and MTX provide indications for their antiproliferative activity on PBMCs and on reduction of intermediate synovial monocytes, both involved in perpetuation of arthritis. While both TNFi and MTX inhibited PBMCs proliferation, only TNFi reduced the intermediate synovial monocytes, suggesting that different immune reactions could be controlled by specific drugs targeting distinct immune populations.

REFERENCES: NIL.

Disclosure of Interests: NIL.