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AB0091 INCREASED BIOLOGICAL AGE IN MALE PARTICIPANTS OF SWEDISH AND UK RHEUMATOID ARTHRITIS COHORTS IS NOT LINKED TO DISEASE

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Background: Immunesenescence in the adaptive immune system, subsequent to thymic involution, results in compromised immunity and increased susceptibility to autoimmune disease and chronic inflammation. There are reports in the literature that immunesenescence, including thymic atrophy and telomere shortening, is accelerated in patients with rheumatoid arthritis (RA)1. What is unclear is whether RA includes accelerated biological ageing overall in addition to immune ageing which may help to explain the increased risk of age-related diseases in RA2. Recent studies have identified a set of DNA methylated sites across the genome that are highly correlated with chronological age and mortality, termed epigenetic clocks3,4 or DNAm age (DNAma), and can be used to determine an individual's biological age.

Objectives: The aim of our study is to determine if the biological epigenetic clocks of RA patients are accelerated.

Methods: We evaluated the Horvath[3] and Hannum[4] epigenetic clocks of control and RA patients using published DNAm data sets, accessions GSE42861 (EIRA, Swedish cohort of 342 RA patients and 328 non-RA controls) and E-MTAB-6988 (77 RA discordant monozygotic twins).

Results: We did not detect significant differences between DNAma of RA and non-RA twins. Similarly, there were no significant differences between the DNAma of RA patients and controls from the Swedish EIRA cohort. However, we detected a significant acceleration in DNAma of male discordant twins, both RA and non-RA, by 5.4 years (p=3.29e-5) and 2.8 years (p=0.04) using the Hannum and Horvath clocks, respectively. Male participants, both control and RA patients, from the EIRA cohort also exhibited an accelerated DNAma, by 1.5 years (p=7.55e-5) using the Hannum clock but using the Horvath clock a significant DNAma acceleration, by 1.4 years (p=0.002) was detected in male RA patients from the EIRA cohort.

Conclusion: Overall, we detected a significant biological age acceleration in male participants from both RA and control groups and only found a significant difference between DNAma of Non-RA controls and RA patients for one of the epigenetic clocks. Further analysis using additional cohort data and biological clock algorithms is needed to confirm our findings.

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AB0092 CTLA4-IG PROMOTES THE M1-M2 SHIFT IN CULTURED MACROPHAGES OF RHEUMATOID ARTHRITIS PATIENTS WITH ACTIVE DISEASE: IN VITRO STUDY

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Background: In rheumatoid arthritis (RA), macrophages play an important role in modulating the immunoinflammatory response through their polarization into "classically" (M1) or "alternatively activated" (M2) phenotypes and the release of pro-inflammatory cytokines (1). In the active inflammatory phase of RA, circulating intermediate monocytes and synovial tissue macrophages show a M1 phenotype, whereas MerTK$^+$M2 macrophages seem to characterize the synovial tissue of RA patients under remission (2-4). In RA, CTLA4-Ig fusion protein (abatacept) reduces the pro-inflammatory activity of macrophages by interacting with the costimulatory molecule CD86 on surface cell membrane of activated cells, including macrophages (2).

Objectives: The *in vitro* study investigated the efficacy of CTLA4-Ig treatment to induce the shift from the M1 phenotype into an M2 phenotype in cultured monocyte-derived macrophages (MDMs) obtained from active RA patients.

Methods: Cultured MDMs obtained from peripheral blood mononuclear cells of 5 active RA patients (mean age 54±13 years) and 5 age-matched healthy subjects (HSs) after overnight stimulation with phorbol myristate acetate (5ng/ml), were treated with CTLA4-Ig at the concentrations of 100mg/mL or 500mg/mL for 3, 12, 24 and 48 hours. A part of cultured RA-MDMs as wells as cultured HS-MDMs were maintained in growth medium (RPMI at 10% of fetal bovine serum) without any treatment and used as unstimulated cells. Gene expression of CD80, CD86 and toll-like receptor-4 (TLR4), as M1 markers, as well as macrophage scavenger receptors (CD163, CD204), mannose receptor-1 (CD206), as surface M2 markers, and MerTK (functional M2 marker) were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Protein synthesis of surface M2 markers was investigated by Western blotting. The statistical analysis was performed by Wilcoxon t-test.

Results: Cultured RA-MDMs showed a high basal gene expression of TLR4, CD80 and CD86 compared to HS-MDMs, confirming to be activated M1 macrophages. In these macrophages, CTLA4-Ig treatment downregulated the gene expression of M1 markers at both concentrations and all timings, but significantly limited to TLR4 and CD80 markers (500mg/mL,12 hours: $p<0.05$). Conversely, both concentrations of CTLA4-Ig significantly upregulated the gene expression of CD163, MerTK and CD206 ($p<0.05$), whereas only the high concentration of CTLA4-Ig significantly upregulated CD204 gene expression ($p<0.05$). The protein synthesis of all M2 surface markers was increased after 24 hours of treatment primarily by the high concentration of CTLA4-Ig, and significantly for CD204 and CD206 ($p<0.05$).

Conclusion: CTLA4-Ig treatment seems to exert an important anti-inflammatory effect by promoting the shift from a M1 to an M2 phenotype in cultured RA macrophages The results suggest a further mechanism for CTLA4-Ig in the modulation of the RA synovitis (5).

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