of DNA DSBs in live mammalian cells triggers the phosphorylation near the C-terminal of H2AX protein, which results in the phosphorylated form of H2AX, termed γH2AX. [1,2].

Objectives: To examine DNA-DSB among patients with systemic autoimmune rheumatic diseases (SARDs) by evaluating phosphorylation γH2AX and 53BP1 at the site of injury.

Methods: The analysis included 25 patients with SARDs (19 with RA (16 women, Me;IQR the disease duration 60 (20-103) months, DAS28-ESR 5.05(4.06-6.9); 4 - with systemic lupus erythematosus (4 women, the disease duration 324 (204-372) months), SLEDAI-2K 9.5 (4.5-16.5) and 2 women with Sjogren’s disease. The control group consisted of 14 healthy donors, comparable in gender and age with the examined patients. DNA-DSB breaks were determined as a discrete focus during immunofluorescence staining with anti-γH2AX and anti-53BP1 antibodies of the lymphocyte culture, followed by analysis on the automated platform AKBIDES (Medipan, Berlin/Dahlewitz, Germany).

Results: There were no significant differences in the number of DNA breaks among patients with SARDs and healthy donors (p=0.05). There was a positive correlation between % pos. cells by γH2AX and CDAI (r=0.45 p=0.035), Nuclei with foci by 53BP1 and ESR (r=0.53 p=0.005) and IgM RF (r=0.63 p=0.005). Among patients with high CDAI activity (n=10), there was a greater number of Nuclei with foci by γH2AX - (22.5 (6.0-43.0); a higher average number of foci mean (0.39 (0.19-0.62) and a higher % pos. cells (32.6 (18.7-39.1) comparing with patients with low/moderate inflammatory activity (9.0 (2-12); 0.12 (0.02-0.28), 11.5 (1.8-23.5) accordingly, p<0.05, n=9).

Conclusion: The evaluation of double-stranded DNA breaks can be beneficial as an additional marker for assessing the disease activity among patients with RA.

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AB0089
INTEGRIN A111 DEFICIENCY AFFECTS THE COURSE OF DISEASE IN THE ARTHRITIC HTNFg MICE

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder conducted by fibroblast-like synovocytes (FLS), which acquire a tumor-like phenotype causing irreversible cartilage and bone damage. FLS attach to the extracellular matrix (ECM) invading the joints, a process mediated by integrins. Integrins are transmembrane proteins regulating several cell functions like cell migration, cell proliferation, tissue invasion and cytokine expression – key mechanisms during the pathogenesis of RA. The collagen-binding Integrin α11β1 (itga11) is expressed on FLS mediating their adhesion to the ECM in this study, we analysed its implication in RA.

Objectives: We examined the pathogenesis of RA in a murine arthritis model (htNFg) lacking α11 to analyze its contribution to joint destructions and the disease course.

Methods: Expression levels of α11 were analysed by Western Blot and immunofluorescence staining using FLS and synovial tissue of patients with RA and hTNFg mice in comparison to their respective controls. Crossbreeding hTNFg with α11 deficient (itga11−/−) mice enables to evaluate arthritis progression using clinical parameters like paw swelling and grip strength. Inflammation area and cartilage damage were quantified by histomorphological techniques such as toluidine blue staining of in paraffin-embedded sections from hind paws. Bone erosion was visualized by μCT imaging and quantification of the bone volume in the tarsal bone area.

Results: High expression levels of α11 could be detected in hTNFg and human RA samples in comparison to their controls. The progression of arthritis in itga11−/−/htNFg was slower and less severe in comparison to hTNFg mice, visible in a stronger grip strength and reduced paw swelling at the same timepoint. The histomorphometry analysis confirmed these results showing higher cartilage area (3.21% vs 5.22%, p < 0.05), less cartilage destruction (5.73% vs 35.65%, p < 0.05) and reduced inflammation (28.28% vs 12.00%) in itga11−/−/htNFg compared to the hTNFg mice. Also, the quantification of the tarsal bone area revealed a higher bone volume (77.94% vs 84.92%, p < 0.01) in itga11−/−/htNFg.

Conclusion: This study showed the important role of α11 in the progression of joint destruction as it is highly expressed in hTNFg mice and in human synovial tissue of patients with RA. Its absence results in less severe arthritis progression and joint destruction, therefore a possible and interesting target for RA treatment.

Disclosure of Interests: None declared.

Background: Immunesenescence is a hallmark of aging and is characterized by a decline in immune function. 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 M2 phenotype macrophages seem to characterize the synovial tissue of RA patients under remission (2). 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) 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 100µg/mL or 500µg/mL for 3, 12, 24 and 48 hours. A part of cultured RA-MDMs as well 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, CD86 and CD206 compared to HS-MDMs, continuing 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 (500µg/mL, 12 hours; p<0.05). Conversely, both concentrations 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 the M1 to M2 phenotype in cultured RA macrophages. These results support a further mechanism for CTLA4-Ig in the modulation of the RA synovitis (5).

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