THU0028

INTERLEUKIN-6 RECEPTOR INHIBITION, AS FIRST-LINE B-DMARD, AFFECTS B CELL SUBPOPULATIONS DISTRIBUTION THROUGH EPIGENETIC MODIFICATIONS IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Despite IL-6R inhibition was found to influence B cell subpopulations distribution in Rheumatoid Arthritis (RA), no data are available on the effect on epigenetic signature of RA B cells by this treatment. It is well known that B cell maturation is under control of the microRNA-155 (miR-155)/PU.1 axis significantly influenced by IL-6 stimulation¹.

Objectives: To investigate the effect of IL-6R inhibition on the epigenetic signature of B cells (miR-155/PU.1 axis) in RA patients.

Methods: Twenty-nine RA patients [18 (62.1%) female; 57.2±14.9 years old; disease duration 1.3±0.7 years] starting IL-6R inhibitor treatment as first b-DMARD, have been enrolled. At study entry and after 3–6–12–18 months follow-up, CD19⁺ cells were isolated from peripheral blood (PB) by magnetic microbeads (Miltenyi) and B cells subpopulations were assessed through FACS according to the IgD/CD27 classification. MiR-155 and PU.1 endogenous expression was determined in PB-derived CD19⁺ cells by RT-PCR at baseline and after 3–6–12–18 months follow-up. IL-6 plasma level was assessed by ELISA at study entry for each patient. ACR/EULAR criteria were used to assess the response rate to IL-6R inhibitor treatment for each RA patient. PB-derived CD19⁺ cells of healthy individuals (HC) were used as comparison group.

Results: At study entry, RA patients showed higher percentage of IgD⁺/CD27⁺ CD19⁺ cells (p<0.05) and IgD⁺/CD27⁺ CD19⁺ cells (p<0.05) than HC. Moreover, IgD⁺/CD27⁺ CD19⁺ cells percentage directly correlated with Disease Activity Score (p=0.04) and IL-6 plasma levels (p=0.06) in RA patients. IL-6R inhibition lead to DAS and SDAI remission achievement in 73.9% and 52.2% of RA patients after 18 months follow-up, respectively, and significantly reduced IgD⁺/CD27⁺ CD19⁺ cells percentage after 18 months follow-up (p<0.02). Stratifying RA patients based on the remission achievement during the follow-up, RA patients who achieved DAS remission under IL-6R inhibition showed a significant decreased of IgD⁺/CD27⁺ CD19⁺ cells percentage compared to patients not achieving this outcome (p<0.05), reaching IgD⁺/CD27⁺ CD19⁺ cells percentage comparable to HC (p>0.05). Analysing the epigenetic profile in B cells of RA patients, at baseline, PB-derived CD19⁺ cells of RA patients showed significantly higher endogenous expression of miR-155 (p=0.04) than HC. Moreover, RT-PCR showed that IL-6R inhibition significantly represses endogenous miR-155 expression in PB-derived RA B cells already after 3 months of treatment (p<0.05) and restores PU.1 expression in PB-derived B cells after 6 months (p<0.05) only in RA patients achieving disease remission.

Conclusions: IL-6R inhibitor, used as first b-DMARD treatment, acts restoring B cells homeostasis through epigenetic modulation in RA. In particular, IL-6R inhibition significantly represses endogenous expression of miR-155 in PB-derived CD19⁺ cells conversely restoring PU.1 expression mirrored by the decrease of IgD⁺/CD27⁺ B cell rate in RA patients achieving disease remission.

REFERENCE:

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THU0029

CTLA-4-IG TREATMENT INDUCES MODULATION OF B-CELL FUNCTION AND DIFFERENTIATION

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Background: CTLA-4 modulates T-cell activation by inducing inhibitory signalling in T-cells. To date the direct effect of CTLA-4-Ig (abatacept) on B-cells has not been studied in detail. We hypothesise that CTLA-4 modulates B-cell function, B-cell homeostasis and B-cell receptor signalling in a T-cell-dependent and T-cell-independent manner.

Objectives: To assess the impact of abatacept on 1. B-cell activation and function *in vitro*; 2. B-cell signalling *in vitro*; 3 maintenance and specificity of memory B-cells specific for citrullinated protein antigens *in vivo*.

Methods: The time kinetic of CD80 and CD86 expression was measured by flow cytometry on isolated B-cells after stimulation via CD40, IL-21R, TLR9, and BCR in presence and absence of CTLA-4-Ig. Human B-cells lines (BJAB) deficient either for CD80 or CD86 were created using the CRISPR Cas9 technology and the effect of abatacept on CD80 respectively CD86 expression was assessed in

these K.O. cell lines. CD80 and CD86 internalisation upon abatacept binding was studied by fluorescence microscopy. The effect of abatacept on PI3K-, NF-κB, PLCγ-signalling and Ca-flux in B-cells was assessed. The effect of CTLA-4-Ig *in vivo* on the pool of memory cells specific for anti-citrullinated protein antigens was studied by limiting dilution assays performed before and during CTLA-4-Ig treatment.

Results: The expression of CD80 and CD86 on isolated human B-cells peaked at day 2–3 of culture after stimulation with CD40-L and IL-21. CTLA-4-Ig led to a decrease in CD80 and CD86 expression. These results were confirmed in human B-cell lines deficient either for CD80 or CD86. By fluorescence microscopy we were able to demonstrate, that abatacept treatment of cultured B-cells leads to a reduction in CD80 and CD86 expression mediated by a mechanism that involves internalisation of the receptors and bound CTLA-4-Ig. To test if CTLA-4-Ig binding activates signalling pathways, we stimulated B-cells with CTLA4-Ig or via BCR or CD40. We detected phosphorylation of p65 indicating activation of the canonical NF-κB pathway. The effect of CTLA-Ig *in vivo* on the pool of memory cells specific for autoantigens (anti-citrullinated protein antigens) was studied by limiting dilution assays in patients with rheumatoid arthritis. This assay is based on polyclonal stimulation of memory B-cells that leads to blast formation and antibody secretion in the supernatant. We performed limiting dilution experiments before and after start of CTLA-4-Ig treatment and observed a significant reduction of the absolute frequency of citrullinated antigen specific memory B-cells in response to treatment.

Conclusions: CTLA-4-Ig-treatment leads to a decrease in B-cells' CD80 and CD86 expression, mediated by a mechanism that involves internalisation of the receptors and bound CTLA-4-Ig. CTLA-4-Ig activates the canonical NF-κB pathway in B-cells. *In vivo* CTLA-4-Ig treatment reduces the frequency of citrullinated antigen specific memory B-cells in patients with rheumatoid arthritis.

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THU0030

MOLECULAR MECHANISMS OF AUTOPHAGIC MEMORY IN PATHOGENIC T CELLS IN HUMAN RHEUMATOID ARTHRITIS

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Background: One of the key elements of immune pathogenesis of human autoimmune arthritis is the resilience of pathogenic T cells. We have previously described that CD4⁺ T cells in patients with arthritis have an increased level of autophagy than their healthy equivalents. Here, we sought to explore at epigenetic and transcriptional levels the concept of persisting increased autophagy as the consequence of "autophagic memory", as one of the mechanisms conferring resilience to pathogenic T cells, in particular to a subset of CD4⁺ T cells (CPL: Circulating Pathogenic-like Lymphocytes), which are significantly more represented in patients with active arthritis and resistant to therapy with biologics

Objectives: To understand molecular mechanism of resilience and persistence in pathogenic T cells in rheumatoid arthritis.

Methods: Autophagy in T cells were analysed using CytoID autophagy detection kit. Jurkat cells pre-starved and control were harvested at various time points, and RNA was extracted for RNA-sequencing and DNA methylation analysis. Illumina paired end sequencing was performed and data was analysed using open source tools in R statistical programming software. CD4⁺ memory and naive T cells were sorted using flow cytometer for qPCR analysis. The CD4⁺ memory and naive cells were sorted using Flow cytometer. RNA extracted and converted to cDNA for qPCR analysis of key genes

Results: First, we demonstrated elevated autophagic levels in CD4⁺ memory T cells when compared to naive CD4⁺ T cells. Second, we showed that autophagic levels are increased in naive and CD4⁺ T cells from RA patients compared to healthy controls. Using next generation RNA-sequencing, transcription factor gene regulatory network (TF-GRN) and methylation analyses, we identified MYC as key regulator of autophagic memory in a human T cell line. Transcriptome and network analysis of RNA-seq data from patients' CPLs confirmed MYC as key modulator of autophagy. Importantly, inhibitor of MYC increases autophagy