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


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. Methods: 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=0.06), reaching IgD-/CD27+ CD19+ cells percentage comparable to HC (p=0.05).

Conclusions: IL-6R inhibitor, used as first b-DMARD treatment, acts restoring B cell homeostasis through epigenetic modulation in RA. In particular, IL-6-R 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.

MEANING OF AUTOANTICORPSES 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-stained 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 tool 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.