SAT0022

THE SUBPOPULATION FEATURES OF DENDRITIC CELLS AS A POTENTIAL BIOMARKER OF EARLY RHEUMATOID ARTHRITIS

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Background: Dendritic cells (DCs) are professional antigen-presenting cells (APCs), which play important role in immune responses. DCs are a heterogeneous population and can be divided into groups: myeloid (mDCs) and plasmacytoid (pDCs). Furthermore, DCs are important in rheumatoid arthritis (RA) pathogenesis through antigen presentation and activation autoreactive T-lymphocytes. As established the different subpopulation DCs can promote different immune reactions. That's why, it may be possible to consider them as a potential target for the development of new target of the immunopathological disorders therapy.

Objectives: To investigate the subpopulations of peripheral blood DCs (myeloid and plasmacytoid) in patients with early RA as a predictor of responsibility to disease-modifying antirheumatic drugs (DMARDs) treatment.

Methods: Forty nine patients with early RA (duration of the disease up to 12 months) were included in the study. All patients fulfilled ACR/EULAR criteria (2010) and received methotrexate, leflunomide, sulfasalazine or their combination. Forty three patients with osteoarthritis (OA) used as a control group. Analysis of the content of the B-lymphocytes, myeloid and plasmacytoid DCs, labeled by antibodies against surface markers, was carried out by flow cytometry. B-lymphocytes, subtypes of peripheral blood DCs were characterized by the following phenotypes: myeloid DCs (CD3-CD14-CD19-HLA-DR + CD11c + CD123-), plasmacytoid DCs (CD3-CD14-CD19-HLA-DR + CD11c-CD123 +), B-lymphocytes (CD19 +). Analysis were performed before treatment and after 3 and 6 months.

Results: Patients with early RA are characterized by significant evaluation of the population of plasmacytoid DCs in comparison of patients with moderate stages of rheumatoid arthritis and osteoarthritis (3.8 vs 2.1 vs 1, p=0.0042). Furthermore, the difference was found in the number of cells with the phenotype B-lymphocytes: $7.95 \times 10^6/l$ vs. $3.6 \times 10^6/l$, respectively (p = 0.014). No significant differences were observed in the number of myeloid DCs. After 6 month of observation we detected reducing amount of plasmacytoid DCs (3.8 before treatment and 2.1 in 6 month, p=0.002) and B-cells that correlated with activity of disease.

Conclusion: The data obtained indicated that plasmacytoid DCs are predominant in patients with inflammatory arthritis especially in early RA and correlate with activity of disease that can use as a predictor of good response on DMARDs treatment.

Disclosure of Interests: None declared

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SAT0023

THE DIFFERENTIAL PRODUCTION OF REACTIVE OXYGEN SPECIES IN T CELL SUBSETS IN PERIPHERAL BLOOD OF RHEUMATOID ARTHRITIS PATIENTS

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Background: T cells play a regulatory role in rheumatoid arthritis (RA) through inducing the homeostasis maintenance and self-tolerance [1]. Specially, the production and the oxidation mechanism of reactive oxygen species (ROS) were out of balance.

Objectives: The aim of the study was to compare ROS productions in T cell subset, which are helper T (TH) cell, cytotoxic T (TC) cell, T helper 17 (TH17) cell and regulatory T (Treg) cell in peripheral blood mononuclear cells (PBMC) of RA patients with RA activity.

Methods: Blood samples were collected from 30 RA patients and 10 healthy adult volunteers under IRB approval. RA activity was divided according to clinical parameter DAS28 [2]. PBMC cells were obtained from the whole blood using lymphocyte separation medium density gradient centrifugation. For separation between the live and dead cell populations, PBMC was stained with Live/Dead stain dye. After PBS washing, cells were incubated with antibodies for CD3, CD4, CD8, and CD25. Following fixation and permeabilization, and further stained with antibodies for FoxP3 and IL-17A. For ROS staining, CellRox and MitoSox were used.

Results: The frequency of TH cell was increased and that of TC cell was decreased in the peripheral blood of RA patients. TH17 and Treg cell population were significantly increased more than about 2-3 folds in active and inactive RA than healthy control. When the whole of cellular ROS production was measured, only Treg cell population was significantly increased in RA than control. Although ROS level was steadily increased with RA activity, there was a slight decline in severe RA compared to moderate and low RA. This difference is larger in mitochondrial specific ROS than total cellular ROS. The mitochondrial complex inhibitor reduced Treg cell frequency in PBMC from RA patients.

Conclusion: Treg is the most sensitive to ROS production among T cell subsets in RA. These findings provide a novel approach to regulate Treg function in RA through mitochondrial metabolism related ROS production.

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SAT0024

TRANSCRIPTOMIC PROFILING OF THE MICROENVIRONMENT DRIVEN RE-SHAPING OF PATHOGENIC CIRCULATORY AND SYNOVIAL HLA-DR+ CD4 T SUBSETS IN ACTIVE JUVENILE IDIOPATHIC ARTHRITIC PATIENTS

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Background: We have previously identified two pathogenic circulatory CD4 subsets in both Teff (CPLs)₁ and Treg (iaTreg)₂ compartments of JIA patients that are HLA-DR⁺, antigen experienced, pro-inflammatory, correlating positively with disease activity and possessing strong synovial TCR sequence coverage. Despite being two functionally discordant T cell subsets (Teff/Treg), their immuno-phenotype and association with clinical fate suggests that these subsets may originate from a common precursor.

Objectives: We seek to understand how the microenvironment could potentially influence and drive these subsets (CPLs/iaTregs) towards their pathological state. In an attempt to elucidate the common pathological gene drivers, we decided to perform next-generation RNA sequencing on sorted CPLs/iaTregs and conventional Teff/Treg counterparts in both circulation and synovium.

Methods: CPLs were sorted as CD3⁺ CD4⁺ CD14⁻ HLADR⁺ CD25/CD127^{low} Teff gate, and iaTregs were sorted as CD3⁺ CD4⁺ CD14⁻ HLADR⁺ CD25^{hi}/CD127^{low} Treg gate with FACs Aria II from n=16 active JIA PBMCs, n=8 paired JIA SFMCs, and n=8 healthy paediatric PBMCs. As a comparative control, similar HLADR⁺ counterparts were respectively sorted from the same patients. Sorted cells were lysed and extracted for RNA, and cDNA conversion/amplification were then carried out using SMART-seq v4. Libraries are prepared and multiplexed using Nextera XT DNA library preparation kit, and ran on the Illumina HiSeq High output platform.

Results: Comparative differential gene expression (DEG) analysis within the circulatory compartment indicate transcriptomic convergence between CPLs/iaTreg and divergence away from conventional Teff/Treg pools. Circulatory CPLs/iaTregs exhibit (a) common pathway dysregulation in T cell signalling (IFN- γ , PD1, CD28 costimulation), (b) restriction in TCR oligo-clonality and (c) common transcription factor drivers (SPL1 and E2F1) within the gene regulatory network, suggesting a common driving source acting on these two disparate compartments.

To understand how this convergence originate, we compared CPLs/iaTreg and conventional Teff/Treg subsets from (a) healthy circulatory PBMCs, (b) JIA circulatory PBMCs and (c) paired JIA synovium. There was a gradual increase in transcriptomic convergence between Teff/CPLs, Treg/iaTreg and CPLs/iaTreg across the spatial/disease continuum, that is paralleled by an antigenic convergence in shared TCR clonotypes in CPLs/