THU0072

GENE SIGNATURE OF PLASMACYTOID DENDRITIC CELLS REVEALS NOVEL PATHWAYS CONTRIBUTING TO TOLERANCE IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Reestablishing immune tolerance and long term remission represent major therapeutic goals in rheumatoid arthritis (RA). Our laboratory previously demonstrated that plasmacytoid dendritic cells (pDCs) from RA patients in remission have the ability to induce IL-10 producing regulatory T cells (Tregs) *in vitro*.¹ However, the molecular pathway of RA pDC-mediated Treg induction remains elusive.

Objectives: Herein, we sought to identify the molecular mechanism through which pDCs contribute to restoration of tolerance in RA.

Methods: pDCs were isolated from peripheral blood of RA patients responding to anti-TNF therapy (remission based on disease activity score DAS28 <5.1) and healthy control subjects and DNA microarrays were performed. Flow cytometry and real time PCR were used to verify the expression of de-regulated genes in RA pDCs. Finally, *in vitro* cultures of pDCs activated with CpG A in the presence or absence of recombinant IL-6 (rIL-6) were performed to assess the functional importance of these gene signatures.

Results: pDCs from RA patients (n=5) exhibited a differential gene signature (6741 deregulated genes) compared to pDCs from healthy controls (n=5). Notably, IL-6 receptor (IL-6R) gene, exhibited increased expression levels in pDCs isolated from RA patients compared to healthy pDCs and the surface expression levels of IL-6 receptor were verified in a subsequent cohort of patients responding to therapy (n=9) versus active patients or healthy donors. Moreover, assessment of IL-6 signalling pathway in RA patients versus healthy donors revealed a significant increase of pSTAT1 expression levels in RA patients (n=9) compared with healthy donors (n=6) (mean fluorescence intensity $\pm$ SEM, 7.98 $\pm$ 0.8 versus 12.65 $\pm$ 1.18, p value=0.0076). Importantly, IL-6-treated pDCs exhibited a vast decrease in TNF- α production (p value=0.0002) whereas no differences were found in the production of IFN- α and in their antigen presenting capacity between CpG-treated pDCs in the presence or absence of rIL-6. Moreover, confocal experiments in progress will assess the expression levels of TNF- α in pDCs isolated from RA patients in remission versus active or healthy donors. The functional importance of the previous findings will be addressed in coculture experiments of IL-6 stimulated pDCs with monocytes isolated from healthy donors and monitor their activation and maturation status.

Conclusions: We found that pDCs from RA patients in remission display increased IL-6R expression levels and an activated IL-6 signalling pathway. Activation of IL-6 signalling on pDCs *in vitro* significantly decreases the production of TNF- α whereas it does not alter IFN- α production and their antigen presenting capacity. This novel finding and the underlying mechanism that may drive pDCs towards a previously described tolerogenic phenotype, need to be further addressed.

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THU0073

A SUBSET OF NEUTROPHIL HIGHLY EXPRESSING CD49D AND VEGFR1 CAN ENHANCE PANNUS FORMATION VIA INCREASE FLS MIGRATION AND INVASION ABILITY BY UP-REGULATING MMP3 AND 13

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Background: Neutrophil is known to play an important role in the progression of rheumatoid arthritis (RA). Pannus formation requires hypoxia microenvironment

(1,2) and a small population of neutrophil highly expressing CD49d, VEGFR1 and CXCR4 is reported to induce angiogenesis at the sites of hypoxia⁽³⁾.

Objectives: In the current study, we aim to identify this subset of neutrophil and investigate its function and role during the pannus formation.

Methods: Collagen-induced arthritis (CIA) model was applied in this study and CD49d⁺VEGFR1⁺Ly6G⁺ neutrophil was monitored at the onset and remission of arthritis. The levels of IL-17, IL-4, IL-6, IL-10, TNF- α and IFN- γ in CIA model sera were tested by ELISA at day 30, 36 and 42, respectively. mRNA expressions of cytokines including TNF- α , VEGF, IL18 and MMP9 were detected by RT-PCR. Meanwhile, chemokines genes of CXCL10, CXCL9, CLL3 and CLL4 expressed in CD49d⁺VEGFR1⁺neutrophil were also measured. *In vitro*, synovial fibroblast-like cells (FLS) was co-cultured with MHL60, expressing CD49d and VEGFR1, and the migration and erosion was measured.

Results: CD49d⁺VEGFR1⁺ neutrophil was detected in the peripheral blood and ankle at various time points with the peak on day 30. The gene expression of TNF- α , VEGF, IL-18 as well as CXCL10, CXCL9, CLL3 and CLL4 was significantly increased in CD49d⁺VEGFR1⁺ neutrophil. Pre-coculture with MHL60 was able to increase FLS migration and erosion. Meanwhile, the expression of MMP13, MMP3 was robustly enhanced at the mRNA and protein level.

Conclusions: A subset of neutrophil highly expressing CD49d and VEGFR1 was detected for the first time in CIA mice. CD49d⁺VEGFR1⁺ neutrophil is able to secrete various chemokine and cytokines. Meanwhile, it can enhance FLS migration and invasion ability via up-regulating MMP3 and 13.

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THU0074

EFFECTS OF RITUXIMAB ON THE INFLAMMATORY AND PRO-THROMBOTIC PROFILES OF NON-B CELLS OF THE IMMUNE AND VASCULAR SYSTEMS OF RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Background: Treatment with B-cell depletion, including rituximab (RTX), has been proven successful in achieving remission from the active disease in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients. However, the effect of RTX on the Cardiovascular disease (CVD) associated to both autoimmune conditions has not been elucidated yet. The aim of this study was to investigate whether B-cell depletion therapy with RTX has an impact on activation of non-B cells of the immune and vascular systems in the context of CVD in RA and SLE patients