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OP0013
LOSS OF SYNOVIAL TISSUE MACROPHAGE HOMEOSTASIS PRECEDES RHEUMATOID ARTHRITIS CLINICAL ONSET

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Background: Synovial tissue macrophages significantly contribute to Rheumatoid Arthritis, yet the precise nature/function of macrophage subsets within the inflamed joint remains unexplored.

Objectives: To fully explore the spectrum of distinct macrophage activation states residing within the synovium of RA, at risk and healthy individuals.

Methods: Single-cell synovial tissue suspensions from RA (n=44), IAR (n=5), HC (n=11), PaA (n=11) and OA (n=4) were obtained, and synovial macrophage subsets examined by advanced multiparameter flow cytometric analysis, bulk RNA-sequencing, metabolic and functional assays.

Results: Multidimensional analysis identifies enrichment of CD206+CD163+ synovial-tissue macrophages co-expressing CD40 in the RA joint compared to healthy synovial-tissue, with frequency of CD206+CD163+CD40+ macrophages associated with increased disease activity and treatment response. In contrast, CX3CR1-expressing macrophages which form a protective barrier in healthy synovium are significantly depleted in RA. Importantly, this signature of enriched CD40 expression coupled with depleted CX3CR1 expression is an early phenomenon, occurring prior to clinical manifestation of disease in individuals ‘at-risk’ of RA (IAR). RNAseq and metabolic profiling of sorted RA synovial-macrophages identified that this population is transcriptionally distinct, displaying unique inflammatory, phagocytic and tissue-resident gene signatures, paralleled by a bioenergetically stable profile as indicated by NAD(P)H emission. Functionally CD206+CD163+ RA macrophages are potent producers of pro-inflammatory mediators (reversed by CD40-signalling inhibition) and induce an invasive phenotype in healthy synovial-fibroblasts. These findings identify a distinct pathological phenotype of synovial-tissue macrophage involved in shaping the immune response in RA. Crucially, this signature is present pre-disease representing a unique opportunity for early diagnosis and therapeutic intervention.

Conclusion: We have identified a novel population of tissue-resident macrophages in the RA synovium which are transcriptionally/metabolically distinct and capable of contributing to disease pathology. Uncovering the molecular patterns and cues that transform this immunoregulatory macrophage population into a dysfunctional inflammatory activation state may provide opportunities to reinitiate joint homeostasis in RA patients.

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OP0014
CTLA4-Ig INDUCES TOLEROGENSIC PROPERTIES OF DENDRITIC CELLS BY ALTERING CELLULAR METABOLISM
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Background: Dendritic cells (DCs) are well-recognized for their dual role either for T cell activation (1) or for inducing T cells tolerance (2). Their ability to modulate T-cell responses has made them an interesting tool for the immunotherapy of autoimmune diseases (3). Cytotoxic T lymphocyte antigen 4 (CTLA4) is a negative co-stimulatory molecule, which binds to CD80/CD86 on DCs. CTLA4 induces its immunoregulatory function through trans-endocytosis resulting in impaired co-stimulation (4), or through the induction of indoleamine-pyrimidine 2,3-dioxygenase (IDO) enzyme (5). Moreover, it has been demonstrated that CTLA4 impairs the autophagic machinery of DCs and therefore suppresses DC inflammatory function (6). Nevertheless, the molecular mechanisms underlying the CTLA4-mediated immunomodulatory phenotype, require a more comprehensive understanding.

Objectives: In this study we focused on tolerogenic DCs (tolDCs) and we applied CTLA4-Ig as a tool to induce them. We aim to assess the immunoregulatory potential of CTLA4-mediated tolDCs and to investigate thoroughly the intracellular pathways that are involved in the induction of tolerance.

Methods: Healthy human monocytes were isolated from peripheral blood and differentiated into monocyte-derived dendritic cells (DCs). After 6 days, immature DCs activated with LPS were treated with CTLA4-Ig or IgG control for 18 hours. The anti-inflammatory function of DCs was validated using RT-PCR and flow cytometry and DCs proliferation to RNA sequencing. The metabolic pathways were studied using a Seahorse bioanalyzer.

Results: CTLA4-Ig-treated DCs showed significantly decreased HLA-DR, CD80/CD86 expression compared to IgG-treated cells (n=4, p=0.0294, n=5 p=0.0079). Moreover, IL6 and TNFα mRNA expression, hallmark of inflammatory cytokines secreted by DCs, was reduced upon CTLA4-Ig (n=5, p=0.0079). To elucidate the pathways involved in DC reprogramming upon CTLA4-Ig treatment, we performed RNA sequencing and we concluded with 1270 differentially expressed genes (p-value <0.05 counts>10). Interestingly, transcriptomic analysis revealed that the majority of genes (n=900) participated in metabolic processes, specifically in OXPHOS pathway and mitochondrial function. To further support the above metabolic changes, we performed Seahorse assays and confirmed that tolDCs had lower basal OXPHOS and decreased ATP production compared with mature DCs. Furthermore, expression of phosphorylated mammalian target of rapamycin (mTOR) and Akt1, central regulators of metabolism, was increased in CTLA4-mediated tolDCs (n=3, p= 0.0308 and p=0.0347).

Conclusion: Herein we confirmed that CTLA4 restricts the pro-inflammatory properties of activated DCs. RNA-seq analysis revealed that this anti-inflammatory deviation of DCs is characterized by the modulation of the expression of genes implicated in cellular metabolism. Metabolic experiments confirmed that CTLA4-mediated tolDCs have reduced OXPHOS and ATP production, whereas, mTOR signaling is upregulated. In future experiments, we will investigate the mechanism that CTLA4 may promote metabolic changes thus contributes to the immunoregulatory phenotype of DCs and could represent a therapeutic target.

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OP0015
PROINFLAMMATORY MONOCYTES AND MACROPHAGES IN SYNOVIAL FLUID AND BURSAL/TISSUE OF PATIENTS WITH POLYMYPALGIA RHEUMATICA: POTENT PRODUCERS OF IL-6 AND GM-CSF
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Background: Polymyalgia rheumatica (PMR) is a common, rheumatic inflammatory disease. Inflammation of bursae and tendon sheaths is a characteristic finding