References:

- [1] Deng J, Wei Y, Fonseca VR, Graca L, Yu D.T follicular helper cells and T follicular regulatory cells in rheumatic diseases[J]. *Nat Rev Rheumatol*. 2019; 15(8):475-490.
- [2] Chen Liu, Dongwei Wang, Songsong Lu, et al. Increased Circulating Follicular Treg Cells Are Associated With Lower Levels of Autoantibodies in Patients With Rheumatoid Arthritis in Stable Remission. *Arthritis Rheumatol*. 2018; 70(5):711-721

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1311

AB0044

ESTABLISHED RHEUMATOID ARTHRITIS PATIENTS HAVE INCREASED FREQUENCIES OF FOLLICULAR REGULATORY T CELLS IN PERIPHERAL BLOOD

C. Tomé¹, S. C. Barreira², P. Martins², A. Valido², R. Barros², J. Polido-Pereira^{1,2}, L. Graca^{1,3}, J. E. Fonseca^{1,2}, R. A. Moura¹. ¹Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; ²Serviço de Reumatologia e Doenças Ósseas Metabólicas, Hospital de Santa Maria, CHULN, Lisbon Academic Medical Centre, Lisbon, Portugal; ³Instituto Gulbenkian de Ciência, Oeiras, Portugal

Background: Several studies have demonstrated that an immune dysregulation affecting both B and T cells occurs in rheumatoid arthritis (RA). Follicular helper T (T_{fh}) cells are crucial for B cell maturation, activation and class-switching as well as for germinal center (GC) formation, whereas follicular regulatory T (T_{fr}) cells can modulate the GC reaction by suppressing T_{fh} and B cells.

Objectives: The main goal of this study was to analyze the phenotype and frequency of circulating follicular T cell subsets in established RA patients.

Methods: Blood samples were collected from established RA patients with active disease, treated with methotrexate (n=32) and from a group of age and sex-matched healthy donors (n=11). Peripheral blood mononuclear cells (PBMC) were isolated and T_{fh} (CD4+CXCR5+CD45RO+) and T_{fr} (CD4+ CXCR5+CD25+FoxP3+) cells, as well as their three major subsets [CXCR3+CCR6- (Th1-like), CXCR3-CCR6- (Th2-like) and CXCR3-CCR6+ (Th17-like)] were evaluated by flow cytometry.

Results: The frequency of circulating T_{fh} cells was similar between established RA patients and controls. Nonetheless, RA patients had a decreased frequency of Th1-like T_{fh} cells, and an increased frequency of Th2-like T_{fh} cells when compared to controls. No significant differences were observed in the frequencies of Th17-like T_{fh} cells between both groups. The frequency of circulating T_{fr} cells was significantly increased in RA patients in comparison to controls. Furthermore, T_{fr} cells from RA patients had significantly increased CD69 median fluorescence intensity (MFI) values when compared to controls. No significant differences were found in the percentages and MFI values of PD-1, ICOS, CD28, CTLA-4, CD40-L and HLA-DR expressed by T_{fh} and T_{fr} cells in RA patients when compared to controls.

Conclusion: Established RA patients have increased circulating frequencies of T_{fr} cells, with higher CD69 expression levels, when compared to healthy controls. These results suggest a pre-activation state of T_{fr} cells in RA and a potential role in the disease pathophysiology.

*RA Moura, JE Fonseca and L Graca are joint senior authors.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5077

AB0045

ULTRASOUND GUIDED BIOPSIES OF RA JOINTS AT TIME OF CLINICAL DIAGNOSIS CONTAIN PROFOUND T CELL CLONALITIES

S. Turcinov^{1,2}, E. Af Klint², A. De Bondt³, M. S. Mia¹, A. Catrina¹, F. Stevenaert³, V. Malmström¹. ¹Karolinska Institute, Division of Rheumatology, Dept of Medicine, Centre for Molecular Medicine, Stockholm, Sweden; ²Karolinska University Hospital, Rheumatology, Stockholm, Sweden; ³Janssen Research & Development, Beerse, Belgium

Background: Rheumatoid arthritis (RA) is a disease characterized by synovial joint inflammation, mainly affecting small joints. Histological findings in synovial biopsies ranges from inflammatory infiltration including ectopic lymphoid structures, to a cell sparse fibroid phenotype. T cells in affected joints are non-naïve and have by flow cytometry approaches been shown to have a wide TCR-beta chain gene usage. New technologies allow for analyses of paired TCR sequences and their antigen-specificities.

Objectives: To study the alpha/beta-T cell receptor repertoire in single sorted T cells from synovial biopsies at time of RA-diagnosis.

Meth ods: Synovial biopsies were taken, primarily using an ultrasound guided technique, from seventeen patients (12 ACPA+, 5 ACPA-) with rheumatoid arthritis. Fresh biopsies were enzymatically digested, followed by mild mechanical treatment, prior to flow cytometry cell sorting. Single cell index sorting of T cells was made into 384-well plates with PCR-buffer followed by a nested PCR

and deep sequencing of the TCR amplicons. TCR-receptor sequences showing clonal expansion from four ACPA+ HLA-DRB1*0401 patients were further cloned into SKW3 cells for studies of their reactivity by *in vitro* stimulation with peptides of viral and citrullinated origin from the literature. A positive response, as measured by CD69-up regulation or IL-2 production, was used to define specificity.

Results: Fourteen of the assessed joints were small (1 MTP, 4 MCP and 8 wrists), whereas the remaining three were large joints (2 knees and 1 ankle), table 1. Individual T cells could be isolated from all of these biopsies, with a varying CD4:CD8 ratio. Based on the flow cytometry phenotyping we could identify CD4 T cells of both Treg and T peripheral helper phenotype already at this early time point. Productive alpha/beta-TCR sequences could be retrieved from 16 out of 17 patients and clonal expansion (>1 copy/TCR) was seen in all but one of these patients, with clone sizes ranging between 2 – 34 copies of each TCR.

Table 1. Patient characteristics.

Patients	Gender (F/M)	HLA-SE alleles	Joints	Joint swelling prior to biopsy (months)	Stiffness specific joint (median VAS)	Pain specific joint (median VAS)
ACPA+ (n = 12)	9/3	*0401, *0404, *0408, *01 and *10	1 MTP, 4 MCP, 6 wrists, 1 knee	4 (1-12) ^a	46 (0-84)	45 (22-99)
ACPA- (n = 5)	3/2	*0401	1 MCP, 2 wrists, 1 ankle, 1 knee	5 (0.25-7)	59 (15-73)	47 (33-81)
SKW3 cell lines (patients n = 4)	4/0	*0401/0404 n=2 *0401 n=2	1 MTP, 3 wrists	2 (1-6)	50.5 (42-84)	50 (40-99)

^a Data not available for one patient. One patient with prior RA-diagnosis, but after 9 months of treatment remission lasting for 20 years.

Artificial T cell lines were generated from the expanded clones of HLA-DRB1*04:01 RA subjects. Our *in vitro* stimulation protocol identified virus specific CD4 T cells in all samples. So far, no citrulline reactivity has been found. HCMV, followed by HHV were the most commonly found viral reactivities, whereas others were found only in one donor (e.g. JCV, EBV). The majority of clones are thus "orphans", to which we are still seeking the driving antigen.

Conclusion: Clonally expanded T cells are found in the synovium of early RA patients and include virus-specific CD4+ T cells. Our data show that the local T cell repertoire is broad already at the time of RA diagnosis

Disclosure of Interests: Sara Turcinov: None declared, Erik af Klint Paid instructor for: Abbvie (courses and lectures), An De Bondt Employee of: Janssen., Muhammad Sohail Mia: None declared, Anca Catrina: None declared, Frederik Stevenaert Employee of: Janssen, Vivianne Malmström Grant/research support from: VM has had research grants from Janssen Pharmaceutica

DOI: 10.1136/annrheumdis-2020-eular.3515

AB0046

NO EVIDENCE FOR DISTURBED TH1 AND TH17 FREQUENCIES IN GCA AND PMR PATIENTS - A STUDY IN THE GRONINGEN GPS COHORT

Y. Van Sleen¹, E. Brouwer¹, M. G. Huijtema¹, W. Abdulahad¹, M. Sandovici¹, A. Boots¹, K. Van der Geest¹. ¹University Medical Center Groningen, Groningen, Netherlands

Background: Giant cell arteritis (GCA) is an aging-associated inflammatory disease of the large-sized arteries. GCA clinically and pathogenically overlaps with polymyalgia rheumatica (PMR), which affects the shoulders and hips. In the inflamed tissues, infiltrated CD4+ T-cells display a disturbed Th cell distribution, that may contribute to the development of the diseases. GCA arteries contain pro-inflammatory T-helper 1 (Th1) and Th17 cells, but almost no Th2 cells or regulatory T-cells. In addition to CD4+ T-cells, macrophages are dominant in GCA and PMR tissue infiltrates. We previously found an altered distribution of monocyte subsets, the precursors of macrophages, in the blood of GCA and PMR patients. Others reported changes in the distribution of Th cells in the blood of treatment-naïve GCA and PMR patients as well.

Objectives: We sought to replicate previous studies showing a disturbed Th cell distribution in the blood of GCA and PMR patients. Next, we aimed to link the disturbed Th cell distribution to the altered monocyte subset counts.

Methods: To assess the capacity of circulating T cells to produce lineage cytokines, PBMCs were cultured for 4 hours in the presence of 50 ng/mL PMA, 1.6 µg/mL calcium ionophore and 10 µg/mL BFA. Cells were then intracellularly stained for IFNγ (Th1 cells), IL-17 (Th17 cells) and IL-4 (Th2) by flow cytometry. In