AB0044

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

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Background: Several studies have demonstrated that an immune dysregulation affecting both B and T cells occurs in rheumatoid arthritis (RA). Follicular helper T (Tfh) cells are crucial for B cell maturation, activation and class-switching as well as for germinal center (GC) formation, whereas follicular regulatory T (Tfr) cells can modulate the GC reaction by suppressing Tfh 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 Tfh (CD4+CXCR5+CD45RO+) and Tfr (CD4+ CXCR5+CD25+FoxP3+) cells, as well as their three major subsets [CXCR3+CXXR6- (Th1-like), CXCR3-CXCR6- (Th2-like) and CXCR3-CXCR6+ (Th17-like)] were evaluated by flow cytometry.

Results: The frequency of circulating Tfh cells was similar between established RA patients and controls. Nonetheless, RA patients had a decreased frequency of Th1-like Tfh cells, and an increased frequency of Th2-like Tfh cells when compared to controls. No significant differences were observed in Tfr cells in these frequencies of Th17-like Tfh cells between both groups. The frequency of circulating Tfr cells was significantly increased in RA patients in comparison to controls. Furthermore, Tfr 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 Tfh and Tfr cells in RA patients when compared to controls.

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

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

Disclosure of Interests: None declared

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AB0045

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

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Background: Rheumatoid arthritis (RA) is a disease characterized by synovial joint inflammation, mainly affecting small joints. Histological findings in synovial biopsies range 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.

Methods: 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 cell 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/TRC) was seen in all but one of these patients, with clone sizes ranging between 2 – 34 copies of each TCR.

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender (F/M)</th>
<th>HLA-SE alleles</th>
<th>Joints</th>
<th>Joint swelling prior biopsy (months)</th>
<th>Specific joint pain (median VAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACyA+</td>
<td>(n=12)</td>
<td>9/3</td>
<td>*0401, *0404, 1 MTP, 4 MCP, 6 wrists, 1 knee</td>
<td>4 (1-12)#</td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>1 MCP, 2 wrists, 1 ankle, 1 knee</td>
<td>5 (0.25-7)</td>
<td>59 (15-73)</td>
<td>47 (33-81)</td>
<td></td>
</tr>
<tr>
<td>SKW3 cell lines</td>
<td>n=2</td>
<td><em>0401</em>0404</td>
<td>1 MTP, 3 wrists</td>
<td>2 (1-6)</td>
<td>50.5 (42-84)</td>
</tr>
<tr>
<td>(patients</td>
<td>n=4)</td>
<td>*0401 n=2</td>
<td>1 MCP, 3 wrists</td>
<td>2 (1-6)</td>
<td>50.5 (42-84)</td>
</tr>
</tbody>
</table>

# 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*0401 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 were 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.

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AB0046

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

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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 T 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. Patients with polymyalgia rheumatica have more monocytes in the blood than healthy controls. In the present study, we aimed to identify the disturbed Th cell distribution in the blood of GCA and PMR patients. 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, 16 μg/mL calcium ionophore and 10μg/mL BFA. Cells were then intracellularly stained for IFNγ (Th1 cells), IL-17 (Th17 cells) and IL-4 (Th2) by flow cytometry. In

References:

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