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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 (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.

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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 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

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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 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 nM 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

addition, monocyte subset counts were determined, based on CD14 and CD16 expression. To obtain absolute counts, proportions determined by flow cytometry were corrected by the total CD4+ T-cell and monocyte counts. Th1, Th17 and monocyte subsets were determined in 21 GCA patients, 19 PMR patients and 19 healthy controls (HC). Th2 cells were determined in 10 GCA patients, 10 PMR patients and 10 HC. All GCA and PMR patients were newly-diagnosed and treatment-naïve. HC were age- and sex-matched and without any immunomodulatory medication.

Results: Both absolute counts and percentages of peripheral Th1 cells, Th17 cells and Th2 cells did not differ between GCA/PMR patients and HC. The monocyte subset in GCA and PMR was mainly attributed to an expansion of the classical monocyte subset. Counts of monocyte subsets were not strongly correlated with counts of either Th1 or Th17 cell counts. In GCA patients, the ESR correlated positively with counts of intermediate monocytes ($R = 0.63$), but this was not observed in PMR patients.

Conclusion: Compared to most previous work, we report similar circulating Th1 and Th17 cell counts in HC, but lower counts in treatment-naïve GCA patients than previously reported (Table 1). Furthermore, numbers of Th1 and Th17 cells in peripheral blood showed no relationship with monocyte subsets. As our protocol for defining Th1 and Th17 cells appears to be similar to the other studies, we propose differences in patient selection. Alternatively, Th1 and Th17 skewing should be studied at the site of inflammation, since Th1 and Th17 skewing cytokines are all highly expressed by macrophages at the inflammatory site. This study shows the importance of replicating previous research, as key concepts of disease pathology are derived from data on disturbed Th cell distribution.

Table 1. Observed median percentages of circulating Th1 and Th17 cells in GCA patients and age-matched HC. Shown are outcomes from several previous studies as well as the present study. Deng et al, Terrier et al and Saadoun et al found elevated Th1 cell percentages in GCA, whereas Samson et al found them lower. All four previous studies showed elevated Th17 percentages in GCA.

Reference	Th1%		Th17%	
	GCA	HC	GCA	HC
Deng et al., <i>Circulation</i> , 2010	20	12	2.2	0.4
Terrier et al., <i>Arthritis Rheumatol</i> , 2012	22	12	2.5	0.5
Saadoun et al., <i>Arthritis Rheumatol</i> , 2015	22	17	2.5	0.6
Samson et al., <i>Arthritis Rheumatol</i> , 2012	10	14	0.7	0.3
This study	11	12	0.6	0.5

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AB0047

T-CELL IMMUNOGLOBULIN AND MUCIN DOMAIN-CONTAINING PROTEIN 3 EXPRESSION IN REGULATORY T CELLS OF ANKYLOSING SPONDYLITIS PATIENTS AND THE CORRELATION WITH DISEASE ACTIVITY

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease. Regulatory T cells have been found in peripheral blood of AS patients. However, there is a controversy regarding the relative number and function of regulatory T cells in AS. T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) is a negative immune regulator that participates in immune responses and widely expresses on a variety of immune cells. Many studies have shown that Tim-3 participates in tumors, infections, hematological system diseases and autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, autoimmune hepatitis, etc). While there has not yet clinical trials with large samples to verify whether or not Tim-3 is involved in the incidence of AS and the relationship between the disease activity of AS and the expression of Tim-3 in regulatory T cells.

Objectives: The present study aimed to identify the Tim-3 expression in regulatory T cells in AS patients, and the association between Tim-3 and the disease activity of AS.

Methods: There were 47 patients diagnosed as AS and 51 age- and sex-matched healthy controls (HCs) from Guangdong Second Provincial Central

Hospital enrolled in the study. The clinical information of the AS patients was recorded in detail and the disease activity was calculated. The positive expression rates and medium fluorescence intensity (MFI) of Tim-3 in regulatory T cells were examined by flow cytometry. Statistical approaches were used to analyze the experimental data of patients and controls.

Results: The Treg cells level was significantly decreased in AS patients compared to the healthy controls (5.14 ± 0.27 vs 4.38 ± 0.23 , $P = 0.032$, Fig 1 A). Tim-3 expression in regulatory T cells was lower in AS patients than the HCs (63.29 ± 2.39 vs 52.56 ± 3.49 , $P = 0.013$, Fig1 B). And Tim-3 MFI in regulatory T cells was significantly decreased too (1355.04 ± 171.44 vs 859.19 ± 105.11 , $P = 0.016$, Fig1 C).

The Treg cells ratio negatively correlated with BASDAI ($r = -0.395$, $p = 0.014$) and BASFI ($r = -0.391$, $p = 0.015$) in AS patients. However, the Tim-3 expression in the Treg cells has no correlation with diseases activity in patients.

Figure 1 The Treg cells frequency, Tim-3 expression and MFI in AS patients compared to the healthy controls.

Conclusion: According to the test results, we could confirm that regulatory T cells participate in the progression of AS, and it has negative relationship with disease activity. Tim-3 can express in Treg, but whether Tim-3 can be used as a potential target for AS treatment in future need further verified.

Disclosure of Interests: None declared

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AB0048

THE EXPRESSION OF T LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD OF PATIENTS WITH AUTOIMMUNE UVEITIS AND THE SHORT-TERM EFFICACY AND SAFETY EVALUATIONS OF LOW-DOSE IL-2 COMBINED THERAPY

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Background: Autoimmune uveitis is a group of inflammatory diseases that affect the uveal tract such as iris, cilia and choroid. In addition to diabetic retinopathy and age-related macular degeneration, uveitis is one of the main causes of blindness in developed countries^[1]. The disease is autoimmune-mediated, and abnormal immune responses are induced by pathogenic antigens such as retinal soluble antigens and retinal interphotoreceptor retinoid-binding protein (IRBP), and autoimmune inflammation is caused by specific cytotoxic effects, immune complex responses and delayed hypersensitivity reactions. It has been found that the disorder of lymphocyte subsets, mainly due to the number and function defects of regulatory T cells (Tregs), may be involved in the development of uveitis. IL-2 is a key cytokine in T cell differentiation. As a new type of immunomodulator, IL-2 has achieved preliminary efficacy in the treatment of systemic lupus erythematosus, ankylosing spondylitis, Sjogren's syndrome and other diseases^[2-4], but there is no clinical evidence of IL-2 in the treatment of autoimmune uveitis. The purpose of this study was to investigate the expression of T lymphocytes in patients with autoimmune uveitis and the effect of low dose IL-2 on their immune status.

Objectives: To investigate the expression of peripheral blood lymphocyte in patients with autoimmune uveitis and evaluate the short-term efficacy and safety of low-dose IL-2 combined with methylprednisolone.

Methods: A total of 108 patients with autoimmune uveitis and 93 healthy subjects who visited our hospital from January 2016 to April 2019 were collected. Twenty-three patients were treated with a low dose of IL-2 (50WU/day for 5 consecutive days) on the basis of conventional treatment