Figure 2. The number of Treg cells and the ratio of Th17/Treg before and after conventional DMARDs therapy.

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SAT0018 THE STUDY OF CD4+T CELL SUBSETS IN RECURRENT POLYCHONDRIITIS

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Background: Relapsing polychondritis (RP) is an rare inflammatory disease of unknown causes, characterized by recurrent inflammation in cartilaginous tissues of the whole body^[1]. The histologic features of the chondritis include loss of basophilic staining of the cartilage matrix, perichondrial inflammation, cartilage destruction with replacement by fibrous tissue, and perivascular cellular infiltration with plasma cells and lymphocytes. Additional clinical features of the disease include ocular inflammation, vasculitis, audiovestibular dysfunction, myocarditis, cardiac valvular insufficiency, and nonerosive inflammatory arthritis. Many studies have shown that the imbalance of helper T cell 17(Th17) and regulatory T cell (Treg) is involved in the pathogenesis of autoimmune diseases such as SLE and RA. But little is known about the roles of peripheral immune cell subsets peripheral in RP patients. Up to now, just few studies focus on this issue.

Objectives: We aimed to analyse the distribution and phenotype of CD4 +T cell subsets in the peripheral blood of patients with RP.

Methods: The proportion and absolute counts of circulating immune cells were assessed in 14 patients diagnosed as RP and 14 healthy controls. CD4+T cell subsets were also analysed in 9 untreated RP patients and 9 healthy volunteers by flow cytometry. All statistical analyses were performed with SPSS v. 22.0. Continuous variables were reported as median. For all study variables, comparison among controls and RP subjects was based on the non-parametric Wilcoxon Mann-Whitney exact test. For all analyses, we used two-sided tests, with p-values <0.05 denoting statistical significance.

Results: Proportion and absolute counts of Treg cells were significantly reduced in RP patients in comparison with controls (proportion, 3.61% vs. 5.24%, p<0.001; absolute counts, 27.36/μl vs. 46.56/μl, p<0.001). But there were no significant difference between the percentage and number of Th17, Th1 or Th2 cells in patients with RP and healthy controls. Thus, the ratio of Th17/Treg increased in RP patients (0.25 vs. 0.14, p<0.001), as did the ratio of Th2/Treg (0.28 vs. 0.22, p<0.001) and Th1/Treg (2.75 vs. 1.92, p=0.019)(Figure 1). Similarly, the proportion and absolute counts of Treg cells in untreated RP patients were significantly lower than that in healthy controls (proportion, 3.78% vs. 5.66%, p=0.008; absolute counts, 32.24/μl vs. 50.76/μl, p<0.001). And the ratio of Th17/Treg also increased in untreated RP patients (0.25 vs. 0.15, p=0.003), as did the ratio of Th1/Treg (2.35 vs. 1.88, p=0.014)(Figure 2).

Conclusion: Our data suggested that the immune-inflammation in RP patients may be related to the depletion of Treg cells and the imbalance of Th17 or Th1 or Th2 and Treg cells. Reduction of peripheral Treg cells may exacerbate the disease progression by not being inhibited Th cells.

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Figure 1: The comparison of CD4+T subsets between 14 patients diagnosed as RP and 14 healthy controls. Compared with healthy control group, the absolute counts of Treg cells of CD4+T cells subsets were significantly reduced in RP patients(p<0.001), so did the Proportion of Treg(p<0.001). But there were no significant difference between the percentage and number of Th17, Th1 or Th2 cells in patients with RP and healthy controls. The ratio of Th17/Treg increased in RP patients (p<0.001), as did the ratio of Th2/Treg (p<0.001) and Th1/Treg(p<0.019).

Figure 2: The comparison of CD4+T subsets between 9 untreated RP patients and 9 healthy controls. Compared with healthy control group, the absolute counts of Treg cells of CD4+T cells subsets were significantly reduced in RP patients(p<0.001), so did the Proportion of Treg(p<0.001). But there were no significant difference between the percentage and number of Th17, Th1 or Th2 cells in patients with untreated RP and healthy controls. The ratio of Th17/Treg increased in RP patients(p<0.001), as did the ratio of Th2/Treg(p<0.019).

SAT0019 CONDITIONED MEDIUM FROM ADIPOSE MESENCHYMAL STEM CELL EXERT IMMUNOSUPPRESSIVE EFFECT ON TH17/TREG AXIS IN RHEUMATOID ARTHRITIS IN VITRO

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Background: Emerging data have demonstrated that mesenchymal stem cells (MSC) are well endowed with a broad repertoire of immunosuppressive tools and they could modulate the immune response on several levels via production of a plethora of soluble factors and contact-dependent mechanisms. For these reasons, mesenchymal stem cells-based protocols were spotlighted as promising multiple-hit treatment candidates for therapeutic intervention in a multitude of autoimmune diseases and debilitating conditions such as rheumatoid arthritis (RA).

Objectives: In line with aforementioned and taking in mind the current shift in Th1/Th2 paradigm suggesting a key role of Th17/Treg axis dysregulations in RA pathogenesis, we aimed to appraise the immunoregulatory effects of secretory factors produced by adipose-derived MSC (AT-MSC) on Th17 and Tregs subsets and also pro-inflammatory cytokines production in patients with RA.

Methods: The study encompasses 17 patients matching the ACR/EULAR 2010 criteria for RA. AT-MSC were isolated and cultured according to well-established protocols. Peripheral blood mononuclear cells (PBMCs) isolated from RA patients' samples were cultured in AT-MSC conditioned media and in control media. Flow cytometry was used for detection of Th17 and Treg markers (of CD3, CD4, CD161, CD196 and CD25 and intracellular expression of FoxP3). ELISA was performed for testing the cytokines produced by PBMC (TGF-β1, TNF-α, IL-17A, IL-21).

Results: We found significant elevation in percentage of CD4⁺CD25⁺FoxP3⁺Treg cell in the pool of PBMC cultured with AT-MSC as opposed to control medium, 4.22 ± 2% (mean ± SD) vs. 6.04 ± 3.6% (p = 0.001). Paired sample analysis of Th17 cells percentage values from all CD4⁺ in PBMC cultured with control media (mean ± SD) 10.13 ± 3.88% in contrast to Th17 percentage in PBMC cultured with AT-MSC supernatants 8.98 ± 3.58% showed significant decrease in Th17 numbers (p = 0.02) under the influence of AT-MSC secretory factors. Cultivation of RA patient's PBMCs with AT-MSC supernatants led to significant up-regulation of TGF-β1 levels, (median [IQR]) 8.318 ng/ml (6.327 – 11.686 ng/ml) vs. 6.227 ng/ml; (1.681 – 10.148 ng/ml) (p = 0.013) and decrease in levels of TNF-α, IL-17A, and IL-21 respectively: 5 pg/ml (1.75 – 11.65 pg/ml) vs. 1 pg/ml (0.7 – 1.9 pg/ml) (p = 0.001); 4.2 pg/ml (3.1 – 6.1 pg/ml) vs. 2.3 pg/ml (0.75 – 5.42 pg/ml) (p = 0.017); 75.1 ± 35.95 pg/ml vs. 52.09 ± 30.8 pg/ml (p = 0.022). The reduction in IL-17 levels positively correlated with the decrease of TNF-α (r = 0.921, p = 0.001).

Conclusion: Our study unambiguously demonstrated that secretory factors produced by AT-MSC exert an immunoregulatory effect on Th17 and Treg cells and also could inhibit major effector cytokine pathways: TNF-α, IL-17, IL-21 production in RA *in vitro*. The study of mechanisms of immunoregulation induced by MSCs provide us better insight into the pathogenesis of rheumatoid arthritis and could be a potential therapeutic approach.

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SAT0020

BLOOD RNA SEQUENCING REVEALS IMMUNOLOGICAL PROCESSES ASSOCIATED WITH THE RESPONSE TO ABATACEPT IN RHEUMATOID ARTHRITIS

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Background: Abatacept (CTLA4-Ig) is an approved biological therapy for the treatment of rheumatoid arthritis (RA). Similar to other biological agents, most patients (60%) respond significantly to this therapy. To date, however, the biological mechanisms underlying the lack of efficacy for this drug are unknown.

Objectives: The objectives of the present study were to characterize the biological processes underlying the lack of efficacy of abatacept and to evaluate the blood transcriptome as a valid source for drug response prediction.

Methods: A total of n=57 patients diagnosed with RA were recruited for this study from 16 rheumatology departments in Spain. All patients were >18 years old and, had >6 months of disease evolution. The primary clinical response to abatacept was defined at week 12 using the EULAR criteria. Good and moderate responders were aggregated into a single response group, and compared to the no response group of patients. Blood RNA was collected from all patients at baseline. From a subgroup of patients (n=31), blood RNA was also obtained at weeks 12, 24 and 48 of treatment with abatacept. Gene expression levels were determined using paired-end RNA-seq (Illumina). Differential gene expression, association to biological processes, longitudinal association analysis and building of the multigenic predictor were performed using the R software and the specialized Bioconductor libraries. The prediction accuracy was evaluated using the ROC AUC.

Results: From the 57 patients treated with abatacept, n=10 (17.5%) were good EULAR responders, n=24 (42%) moderate EULAR responders and n=23 (40.5%) non-responders at week 12 of therapy. Biological process analysis identified two significantly distinct biological profiles between responders and non-responders. In responders, we found an association to pathways associated with the effector phase of T cells (e.g. interleukin-15 and 2 signalling, P < 0.05). Non-responder patients showed instead a strong association to biological processes associated with antigen presentation and activation of T cells (P < 0.005). Using the baseline gene expression profiles, we built a multigenic predictor of response to abatacept with an AUC = 75%. In the longitudinal cohort, patients were stratified based on reaching an inactive state (i.e. DAS28 < 3.2). Using this endpoint measure, the longitudinal analysis of the 4 time points corroborated the association of response with antigen presentation (P < 0.01).

Conclusion: The analysis of blood RNA profiles of RA patients has enabled the identification of specific biological processes associated with the lack of response to abatacept. Also, we demonstrate that blood expression profiles can be predictive of the response to the drug at week 12 of therapy.

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SAT0021

ELEVATED NUMBERS OF C-TYPE LECTIN CD161 POSITIVE PR3-SPECIFIC T-CELLS IN GPA

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Background: Various alterations of the peripheral T-cell compartment have been reported in granulomatosis with polyangiitis (GPA) such as elevated populations of CD4+CD8+ double-positive and CD4+CD161+ and CD28- single-positive effector memory T-cells (T_{EM}) within the total CD3+ T-cell population (1). Analysis of antigen-specific T-cell subpopulations shows, that PR3-specific T-cells display Th2-type, Th17 and Th22 cytokine profiles in GPA (2). Moreover, concomitant cellular CMV- and Epstein Barr virus (EBV)-infection has been found to be associated with the expansion of CD28- T_{EM} in GPA (1,2). Notably, C-type lectin CD161 +CD8+ T-cells displaying a polyfunctional memory profile directed against several common viruses have been reported. Furthermore, CD161+ T-cells are involved in the pathogenesis of early stage autoimmune hepatitis (3). CD161 expression on proteinase 3 (PR3)-specific T-cells in comparison to other antigen-specific T-cells has not been described in GPA as yet.

Objectives: To determine the amount of C-type lectin CD161 on antigen-specific CD8+ single-positive and CD4+CD8+ double positive T-cells in patients with GPA

Methods: In this study, we analyzed the expression of CD161 and CD28 on circulating antigen-specific CD8+ single-positive and CD4+CD8+ double positive T-cells in HLA-A2 positive patients with GPA (n=21) and healthy controls (n=21) using flow cytometry. Antigen-specific T cells were detected using peptide/MHC class 1 dextramers containing major peptide epitopes for PR3, Epstein Barr virus (EBV) BMLF1, and Cytomegalovirus (CMV) pp65 (aa 196-177, aa 280-288, and aa 495-504, respectively).

Results: Patients with GPA showed a higher frequency of circulating CD8 + single-positive and CD4+CD8+double-positive PR3-specific T-cells with increased expressions of CD161 compared to HC. Compared to EBV- or CMV-specific T-cells, there was an increased expression of CD161 on PR3-specific T-cells in GPA. In contrast to HC and EBV- or CMV-specific T-cells, the percentage of CD28+ T-cells was expanded within the PR3-specific CD8+ T-cell subset in GPA.

Conclusion: These findings suggest a potential role of CD161 as an additional TCR-independent co-stimulatory receptor on PR3-specific T-cells in GPA. The role of these cells in the pathophysiology and as a potential therapeutic target remains to be further investigated.

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