THU0044 IMPAIRED AUTOPHAGY INDUCES DEFECTIVE FUNCTION OF REGULATORY B CELLS IN ANKYLOSING SPONDYLITIS

C. Shuizhong, X. zhongyu, Z. guan, S. huiyong, W. yanfeng. *Department of Orthopedics, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China*

Background: Ankylosing spondylitis (AS) is an autoimmune disease characterised by pathological osteogenesis and chronic inflammation. Large number studies shown that Regulatory B cells (Bregs) has immunosuppressive function, which could be involved in many rheumatic disease, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA). But the Bregs in AS are poorly understood.

Objectives: To investigate the ratio and function of Bregs in AS, and illuminate the under lying mechanism, which might help to further understand the pathology of AS.

Methods: (1) Peripheral blood mononuclear cells (PBMCs) were collected from 9 AS patients and 9 healthy controls, then the Bregs were detected by using flow cytometry with the following antibodies: CD19-PE, CD24-FITC and CD38-APC.² B cells were purified with a CD19 magnetic bead, the Bregs were sorted by using the flow cytometry. Bregs were added to the upper chamber with 1.5 ml medium, while CD4 + T cells were added to the lower chamber with 2.6 ml medium at a ratio of 1:1 Bregs(1 × 10⁶ cells): CD4 + T cells(1 × 10⁶ cells). CD4 + T cells were incubated with 5 μM CFDA-SE. The CD4 + T cell proliferation was analysed in the fifth day.³ The cytokines of Bregs were detected with a proteome profiler kit, and confirmed by using ELISA and Western Blot.⁴ We detected the Bregs autophagy between AS and HD. And we regulate the autophagy with rapamycin and 3-methyladenine (3-MA), then detected the IL-10 secretion of Bregs and the CD4 + T cell proliferation among groups.

Results: (1) The ratio of Bregs in AS was higher than in healthy group.² Bregs from AS shown a impaired function in suppressed the CD4 + T cell proliferation compared with HD.³ Bregs of AS expressed and secreted less IL-10 than healthy control, and might explain the impaired function of Bregs of AS.⁴ Exogenous IL-10 recovered the immunosuppressive capacity of AS Bregs, whereas exogenous anti-IL-10 antibody reduced the immunosuppressive capacity of HD Bregs.⁵ Autophagy impaired in AS Bregs compared with HD Bregs. Induce autophagy in AS Bregs could increase the IL-10 secretion and strengthened its immunosuppressive capacity, while 3-MA shown the opposite results.

Conclusions: Even increasing ratio of Bregs in AS, but they had a impaired function in suppressed CD4 + T cell proliferation compared with the HD. We further found that impaired autophagy could induces less IL-10 secretion, which further affected the immunosuppressive capacity of Bregs of AS.

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THU0045 EXPANSION OF ACTIVATED CXCR5+ICOS+ TFH CELLS AND PLASMA BLASTS INDUCED BY SEASONAL INFLUENZA VACCINE IS IMPAIRED IN ANTI-IL-6R TREATED RHEUMATOID ARTHRITIS PATIENTS

V.C. Romão^{1,2}, A. Agua-Doce³, J. Polido-Pereira^{1,2}, R. Barros², I.P. Lopes¹, M. I. Seixas², M.J. Saavedra², E. Sacadura-Leite⁴, H. Rebelo-de-Andrade^{5,6}, J. E. Fonseca^{1,2}, L. Graca³. ¹Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa; ²Rheumatology Department, Hospital Santa Maria-CHLN; ³Lymphocyte Regulation Lab, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa; ⁴Occupational Medicine Department, Hospital Santa Maria-CHLN; ⁵Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, IP; ⁶Host-Pathogen Interaction Unit, Research Institute for Medicines (iMed. ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Background: T follicular helper (Tfh) cells are essential for the generation of high affinity neutralising antibodies elicited following vaccination and are involved in the pathogenesis of rheumatoid arthritis (RA). Interleukin (IL)-6 has been shown to be critical for Tfh differentiation in mice, while its importance in humans has been less clear, given the lack of adequate *in vivo* assessment.

Objectives: To investigate the importance of IL-6 for the *in vivo* differentiation of human Tfh cells, taking advantage of influenza vaccination in patients under anti-IL-6R therapy.

Methods: Blood was collected before, 7 and 28 days after vaccination from established RA patients treated with tocilizumab (TCZ, IL-6R blocker), methotrexate (MTX) ± other DMARDs and age- and sex-matched healthy donors (HD). We analysed the frequency of Tfh (CD3⁺CD4⁺CD25⁺Foxp3⁺CXCR5⁺CD45RO⁺), T follicular regulatory (Tfr, CD3⁺CD4⁺CD25⁺Foxp3⁺CXCR5⁺) and B cell populations at each time point. We used non-parametric tests, deemed significant at p<0.05.

Results: We included 137 participants (42 TCZ, 42 MTX, 53 HD) with similar age and gender distribution. Patients from the TCZ group had more active and severe disease. At baseline, patients treated with TCZ had higher frequency of Tfh and Tfr-Th2-like cells (CXCR3⁺CCR6⁺) and lower frequency of Tfr-Th1-like (CXCR3⁺CCR6⁺) and B cells. Following influenza vaccination, the overall blood Tfh and Tfr populations remained unchanged in all groups. However, as previously reported, there were marked changes in specific subsets at day 7 of HD following vaccination. We found a marked expansion of activated CXCR5⁺ICOS⁺ Tfh cells at day 7, in HD and MTX-treated patients, but this was impaired in the TCZ group (figure 1). The increase in activated CXCR5⁺ICOS⁺ Tfh cells was mainly due to a Tfr-Th1-like subpopulation, greatly increased in HD and MTX-treated patients (figure 1). Of note, CXCR5⁺ICOS⁺ Tfr-Th1-like cells also accumulated in HD but not in RA patients. The proliferative capacity of CXCR5⁺ICOS⁺ Tfh cells seemed to be partially impaired in patients under IL-6R blockade, that displayed marked reduction of Ki67⁺CD38⁺ proliferative cells within that compartment (figure 1). Anti-IL-6R treatment also impaired expansion of CD19⁺IgD⁺CD27⁺CD38^{hi} plasmablasts following vaccination, when compared with both MTX and HD groups (figure 1). Changes in CXCR5⁺ICOS⁺ Tfh and plasmablasts were significantly correlated in all groups.

Abstract THU0045 – Figure 1. Frequency of cell populations within the CD4+ in the blood. All tests are paired and non-parametric. Healthy N=53, MTX N=42, TCZ N=42.