

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

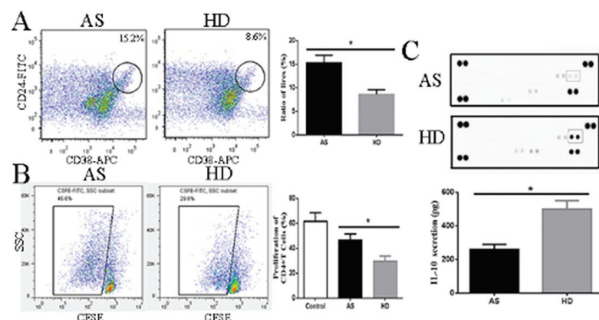
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Background: Ankylosing spondylitis (AS) is an autoimmune disease characterised by pathological osteogenesis and chronic inflammation. Large number studies shown that Regulatory B cells (Bregs) has immunosuppressive function, which could be involved 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 analyse in the fifth day.³The cytokines of Bregs were detected with a proteome profiler kit, and confirmed by using Elias 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 that 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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Disclosure of Interest: None declared

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

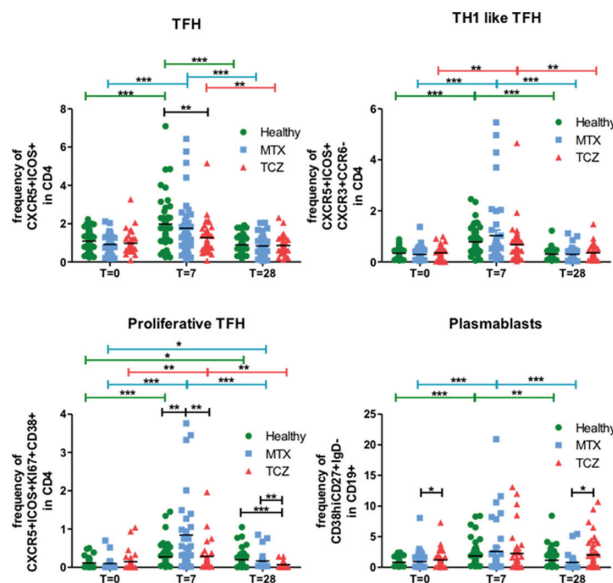
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Background: T follicular helper (Tfh) cells are essential for the generation of high affinity neutralising antibodies elicited following vaccination and are involved in the pathogenesis of rheumatoid arthritis (RA). Interleukin (IL)-6 has been shown 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 Tfh-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 Tfh-Th1-like subpopulation, greatly increased in HD and MTX-treated patients (figure 1). Of note, CXCR5⁺ICOS⁺ Tfh-Th17-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.

Conclusions: Anti-IL-6R treatment limits proliferative ability of activated CXCR5⁺ICOS⁺ Tfh cells, blocking their emergence as well as plasmablast accumulation following influenza vaccination. Our data suggest that IL-6 is crucial for optimal *in vivo* generation of activated Tfh cells in humans.

Disclosure of Interest: None declared

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THU0046 SMALL MOLECULE INHIBITOR OF THE WNT PATHWAY (SM04755) AS A POTENTIAL TOPICAL TREATMENT FOR PSORIASIS

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Background: Psoriasis (PSO) is an autoimmune disease, causing patches of thick, inflamed, scaly skin due to excessive proliferation of skin cells¹. Wnt signaling plays an important role in PSO, regulating inflammation and keratinocyte proliferation. SM04755, a novel, topical small-molecule Wnt pathway inhibitor was previously shown to inhibit inflammation and keratinocyte proliferation *in vitro* and in an IMQ-induced mouse PSO model².

Objectives: In this study, the effects of SM04755 on inflammation and skin health were evaluated in two models that closely resemble human PSO pathophysiology: reconstitution of ICR *scid* mice with minor histocompatibility mismatched naïve CD4⁺ T lymphocytes³ and an IL-23 intra-dermal injection model⁴.

Methods: For (A) immune reconstitution model, peripheral blood mononuclear cells were isolated from F₂ (BALB/c x 129/SvJ) mice and analysed by flow cytometry to identify H-2D^d haplotype donor mice. CD4⁺/CD45RB^{Hi} cells from donor mice spleens were purified and injected intravenously into CB17/ICR-Tac Prkdc/*scid* (ICR *scid*) mice (5 × 10⁵ cells/mouse). Skin appearance and ear thickness were evaluated weekly. At the first visible PSO-like signs, mice were randomised and treated with SM04755 (400 µg/cm²) or vehicle. After 14 weeks, body and spleen weights were measured, and inflammation was evaluated by measuring cytokines (IL-1β, TNF-α, IL-6) in tissues from skin, ears, spleen and plasma using ELISA. Epidermal thickness and skin immune cell infiltration were histologically evaluated. For (B) the IL-23 model, rIL-23 was injected intra-dermally into mouse ears, every other day for 35 days. Mice were randomised on Day 16 and treated with SM04755 (400 µg/cm²) or vehicle or Clobetasol daily for 20 days. Ear thickness was measured every 3 days. Skin immune cell infiltration was histologically evaluated.

Results: (A) Immune reconstitution of ICR *scid* mice resulted in PSO-like signs, with skin lesions and increased thickness of the skin and ears. Treatment with topical SM04755 (400 µg/cm²) significantly (p<0.01) decreased skin and ear thicknesses and improved skin appearance compared to vehicle. Body weights were significantly (p<0.05) higher in treated compared to vehicle mice. SM04755 significantly reduced histologically measured epidermal thickness (p<0.05) and immune cell infiltration in the skin compared to vehicle. Further, inflammatory cytokine levels in the skin, ears, spleen and plasma and spleen weight were significantly (p<0.05) reduced in SM04755 treated animals compared with vehicle. (B) Intra-dermal IL-23 injection into mouse ears resulted in inflammation and ear thickening by day 16 compared to sham. Treatment with topical SM04755 (400 µg/cm²) significantly (p<0.05) decreased ear thickness, immune cell infiltration, and improved appearance compared to vehicle.

Conclusions: In two mouse models of (A) minor histocompatibility mismatched T lymphocyte reconstitution-induced PSO and (B) IL-23 injection-induced PSO, topically applied SM04755 inhibited key pathophysiological features of PSO at macro- and microscopic levels, compared to vehicle. SM04755 has potential as a topical therapy for PSO. Clinical trials are ongoing.

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Disclosure of Interest: V. Deshmukh Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, M. Pedraza Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, L. Lamangan Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, Y. Yazici Shareholder of: Samumed, LLC, Employee of: Samumed, LLC

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THU0047 1,25(OH)2D3 AND DEXAMETHASONE ADDITIVELY SUPPRESS SYNOVIAL FIBROBLAST ACTIVATION BY CCR6+ TH MEMORY CELLS AND ENHANCE THE EFFECT OF TNF-ALPHA BLOCKADE

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Background: Despite improvement in treatment of rheumatoid arthritis (RA) over the past decades, insufficient treatment response and treatment resistance in many patients demonstrate the need to develop new therapeutic strategies. Chronic synovial inflammation could be suppressed by targeting activation of RA synovial fibroblasts (RASf) by for example IL-17A-producing CCR6 +T helper memory (memTh) cells. Previously, we have shown that dexamethasone (DEX) combined with the active vitamin D metabolite 1,25(OH)₂D₃ reduces pathogenicity of memTh cells.

Objectives: To study the additive effect of 1,25(OH)₂D₃ and DEX on suppressing the pro-inflammatory loop between RASf and CCR6 +memTh cells and explore potential therapeutic applications.

Methods: CCR6 +memTh cells from PBMC of healthy donors or treatment-naïve early RA patients were cultured alone or with RASf from established RA patients for three days and treated with or without 1,25(OH)₂D₃, DEX or etanercept. Treatment effects were assessed using ELISA and flow cytometry.

Results: CCR6 +memTh produces less of the pro-inflammatory cytokines IL-17A, IL-22 and IFNγ upon exposure to 1,25(OH)₂D₃, and to a lesser extent by DEX. TNFα was only inhibited by the combination of 1,25(OH)₂D₃ and DEX. In contrast, in RASf cultures DEX was the strongest inhibitor of IL-6, IL-8 and tissue-destructive enzymes. As a result, 1,25(OH)₂D₃ and DEX additively inhibited inflammatory mediators in CCR6 +memTh RASf co-cultures. Interestingly, low doses of mainly DEX, but also 1,25(OH)₂D₃, combined with etanercept better suppressed synovial inflammation in this co-culture model compared to etanercept alone.

Conclusions: This study suggests that 1,25(OH)₂D₃ and DEX additively inhibit synovial inflammation through targeting different pro-inflammatory mechanisms. Furthermore, low doses of DEX and 1,25(OH)₂D₃ enhance the effect of TNFα blockade in inhibiting RASf activation, providing a basis to improve RA treatment.

Disclosure of Interest: None declared

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THU0048 PRO-INFLAMMATORY IL-17A-PRODUCING CCR6+ T HELPER MEMORY CELLS CHANGE INTO ANTI-INFLAMMATORY CELLS WITH REGULATORY CAPACITY UPON EXPOSURE TO ACTIVE VITAMIN D

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Background: In autoimmune diseases such as rheumatoid arthritis (RA), an important therapeutic goal is to normalise the imbalance between pro- and anti-inflammatory cells. In RA, especially pro-inflammatory CCR6 +T helper (Th) memory cells, characterised by IL-17A production and RORC expression, are elevated and more activated compared to healthy controls. Therefore, modulating these cells to become anti-inflammatory could contribute to restoring the immunological balance. Interestingly, the active vitamin D metabolite 1,25(OH)₂D₃ inhibits pro-inflammatory cytokine production by CCR6 +Th memory cells.

Objectives: We investigated whether 1,25(OH)₂D₃ can induce an anti-inflammatory phenotype in these memory CCR6 +Th cells.

Methods: CCR6 +Th memory cells, excluding Tregs, were sorted from treatment-naïve early RA patients or healthy controls and cultured with or without 1,25(OH)₂D₃. Effects were analysed using microarray, RT-PCR, ELISA or flow cytometry. Functional properties were assessed via suppression and chemotaxis assays.

Results: 1,25(OH)₂D₃ inhibits pro-inflammatory cytokines such as IL-17A, IL-17F and IL-22 in CCR6 +Th memory cells from both healthy controls and RA patients. This is accompanied by induction of anti-inflammatory factors, including IL-10 and CTLA4. Interestingly, these formerly pathogenic cells suppress proliferation of autologous CD3 +T cells, similar to classical Tregs. Importantly, the modulated memory cells still migrate towards the site of inflammation, modelled by synovial fluid, and retain their suppressive capacity in this environment.