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 show 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 underlying 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. 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(1×10^6 cells): CD4 + T cells(1×10^6 cells). CD4 + T cells were incubated with 5 μM CFDA-SE. The CD4 + T cell proliferation was analysed in the fifth day. The cytokines of Bregs were detected with a proteome profiler kit, and confirmed by using Elias and Western Blot. We detected the Bregs autophagy function by using autophagy marker LC3 levels and dynamin receptor 1 (DHR1) level.

Results: (1) The ratio of Bregs in AS was higher that in healthy group. 2 Exogenous IL-10 recovered the immunosuppressive capacity of AS Bregs, whereas exogenous anti-IL-10 antibody reduced the immunosuppressive capacity of HD Bregs. 3 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 induce less IL-10 secretion, which further affected the immunosuppressive capacity of Bregs of AS.

Acknowledgements: This study was supported by the National Natural Science Foundation of China (81271951, 81401850), the Science and Technology Project of Guangdong Province (2015 B020228001) and by the Engineering Technology Research Centre for Comprehensive Diagnosis and Treatment of Ankylosing Spondylitis of Guangdong Higher Education Institutes (GCZX-A1301).

Disclosure of Interest: None declared

THU0045

EXPANSION OF ACTIVATED CXCR5+ICOS+ T FH 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) the follicular regulatory (Trf, CD3 CD4 CD25 Foxp3CXCR5) 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 Th2-like cells (CXCR3CCR6) and lower frequency of Trf-Th1-like (CXCR3CCR6) and B cells. Following influenza vaccination, the overall blood Tfh and Trf 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 Thf-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).

Conclusions: Anti-IL-6 treatment also impaired expansion of CD19 IgD+ CD27 CD38 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.
SMALL MOLECULE INHIBITOR OF THE WNT PATHWAY
(SM04755) AS A POTENTIAL TOPICAL TREATMENT
FOR PSORIASIS

THU0046

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Background: Psoriasis (PSO) is an autoimmune disease, causing patches of thick, inflamed, scaly skin due to excessive proliferation of skin cells. 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 naive CD4+ T lymphocytes and an IL-23 intra-dermal injection model4.

Methods: For (A) immune reconstitution model, peripheral blood mononuclear cells were isolated from F2 (ALB/c x 129/SvJ) mice and analysed by flow cytometry to identify H-2Dd haplotype donor mice. CD4+CD45RB+ cells from donor mice spleens were purified and injected intravenously into CB17/ICR-Tac Prkdc/scid (ICR scid) mice (5 x 10^7 cells/mouse). Skin, ear, and spleen appearance compared to vehicle. Further, inflammatory cytokine levels were assessed in plasma and serum. SM04755 (400 μg/cm^2) was topically applied for 35 days. Mice were randomised on Day 16 and treated with vehicle or SM04755 (400 μg/cm^2) daily for 20 days. Ear thickness was measured every 7 days. Skin, ear, and spleen skin thickness were measured by histology. For (B) the IL-23 model, IL-23 was injected intra-dermally into mouse ears, every other day for 35 days. Mice were randomised on Day 16 and treated with vehicle or SM04755 (400 μg/cm^2) daily for 20 days. Ear thickness was measured every 7 days. Skin, ear, and spleen skin thickness were measured by histology.

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/cm^2) significantly (p<0.05) 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 were significantly reduced in the skin, ears, spleen and plasma of vehicle treated mice compared to vehicle. At the first visible PSO-like signs, mice were randomised and treated with SM04755 (400 μg/cm^2) or vehicle. After 14 weeks, body and spleen weight were significantly (p<0.05) higher in treated compared to vehicle mice. SM04755 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 (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 (p<0.05) reduced in SM04755 treated animals compared with vehicle. Further, inflammatory cytokine levels and cytokines (IL-1β, TNFα, IL-6) in tissues from skin, ears, spleen and plasma using ELISA. Epidermal thickness and skin cell infiltrate were histologically evaluated. For (B) the IL-23 model, IL-23 was injected intra-dermally into mouse ears, every other day for 35 days. Mice were randomised on Day 16 and treated with vehicle or SM04755 (400 μg/cm^2) or vehicle. After 14 weeks, body and spleen weight were significantly (p<0.05) higher in treated compared to vehicle mice. SM04755 significantly (p<0.05) decreased ear thickness, ear 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.

REFERENCES:


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 cells treated with 1,25(OH)2D3 or DEX equally significantly reduced pro-inflammatory cytokines in CCR6+ memTh control cells. 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


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 inflammatory cytokines in 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.