among ILCs. Multivariate linear regression and Receiver-Operating Characteristic (ROC) Curve analysis was performed using the IBM SPSS Statistics software. Different in vivo models were used to assess functional implications of ILCs at different time points of the disease. Joint inflammation was assessed through MRI and H&E staining of ankle areas. Peripheral blood was obtained from mice of each group and flow cytometry analysis was performed. High dimensional analyses including RNA-seq was performed to identify phenotypic characteristics of ILCs implemented into the pathogenesis of the disease.

Results: Total number of circulating ILCs were increased in PsA patients compared to PsO and healthy controls (p<0.001). Linear regression analyses of the relationship between disease activity and circulating ILCs counts showed strongest correlation between ILC3s counts and DAPSA score. ILC3s counts also correlated with imaging signs of inflammation such as enthesitis, synovitis, erosions and/or osteoporosis as assessed by MRI and H-pQCT. Musculoskeletal inflammation in mice was predominantly associated with p19 expression and IL-23R-signaling as assessed by RNA-seq. These effects were also accompanied by a strong upregulation of IL-17-producing lymphocytes within the inflamed joint niche with a dominant presence of ILC3s. Multi-channel immunofluorescence and confocal laser scanning microscopy revealed not only upregulation of ILC3 induced IL-17 production within the synovial membrane but also in peri-articular areas of the inflamed joints.

Conclusion: ILC3s not only correlate with various facets of PsA manifestations but also functionally contribute to synovitis and enthesitis suggesting them as an interesting target for upcoming treatment strategies in the near future.

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WNT SIGNALING CAN PLAY AN IMPORTANT ROLE IN VASCULAR CALCIFICATION IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background: Vascular calcification is highly correlated with atherosclerosis. Ankylosing spondylitis (AS) is associated with a process of accelerated atherosclerosis. Wnt signaling plays an important role in the pathogenesis of vascular calcification. However, there has been no study of the role of Wnt signaling in vascular calcification in patients with AS.

Objectives: We investigated the relationship between vascular calcification and Wnt signaling in patients with AS.

Methods: Sixteen male patients aged over 20 years with AS were enrolled. They fulfilled the modified New York criteria and each of their ankylosing spondylitis disease activity score was more than 2.1. Sex and age matched nineteen healthy controls were also recruited.

Mouse MOVAS vascular smooth muscle cell line (American Type Culture Collection, ATCC® CRL-2797™) were stabilized in maintain media for 24 hours. Then media were exchanged for the 10% serum of patients with AS or controls in maintain media. Cells were stimulated for another 72hours. We exchanged this medium with calcification medium. Cells were cultured until 2 weeks then stained with Alizarin Red S and the optical density (OD) was measured.

For Western blotting and RT-qPCR, cells were stabilized for 24 hours and stimulated for another 72 hours through the same procedure as that of Alizarin Red S staining. After cell stimulation, the level of mRNA and protein were measured by RT-qPCR and western blot, respectively. We measure the level of Low-density lipoprotein receptor-related protein (LRP)5, LRP6, Dickkopf-related protein 1, Wnt3a, matrix metalloproteinase-7 (MMP-7), beta-catenin for canonical Wnt signaling; Receptor Tyrosine Kinase Like Orphan Receptor 2, Wnt5a, Runt-related transcription factor 2 (RUNX2) for non-canonical Wnt signaling. We also checked the level of Alkaline phosphatase (ALP), IL-17, IL-23 and TNF-a.

Results: The level of OD of MOVAS cells treated with serum from AS patients (10.503 ± 4.622, mean ± SD) was significantly higher than that from controls (10.994 ± 4.291) (P=0.000, Mann-Whitney test). The protein level of MMP-7 and beta-catenin of MOVAS cells treated with serum from AS patients (1.881 ± 0.687; 1.301 ± 0.342) was significantly higher than that from controls (0.779 ± 0.48; 0.854 ± 0.285) respectively (P=0.005; P=0.002, Mann-Whitney test). The mRNA level of RUNX2, ALP, IL-17 and IL-23 of serum from AS patients (2.567 ± 1.46; 2.687 ± 1.753; 2.253 ± 1.129; 0.574 ± 1.142) was significantly higher than that from controls (1.557 ± 0.587; 1.696 ± 0.637; 1.358 ± 0.473; 1.386 ± 0.714) respectively (P=0.000; P=0.037; P=0.044; P=0.007, Mann-Whitney test). There was a binary positive correlation between the mRNA level of WNT5a and RUNX2 (r=0.705, p=0.002, Spearman rank correlation coefficient) and the protein level of WNT5a and ALP; MMP-7 and TNF-a; MMP-7 and IL-17; MMP-7 and IL-23 (r=0.601, p=0.039; r=0.769, p=0.026; r=0.828, p=0.011; r=0.777, p=0.003), respectively.

Conclusion: 1. Vascular smooth muscle cell calcification was increased in patients with ankylosing spondylitis than those of the control group.

2. The level of several molecules (i.e. Beta-catenin, RUNX2, MMP-7) related to Wnt signaling of vascular smooth muscle cells treated with serum of patients with AS was elevated significantly compared to those of controls and positively related.

3. Wnt signaling can play an important role in vascular calcification in patients with ankylosing spondylitis.

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of several disease relevant mediators such as TNF, IL-23 and CCL20 in both immune and stromal lineage cells. This is the first demonstration of IL-36 production in human enthesis. Given its pleiotropic effect and relation to IL-23/IL-17 axis, IL-36 is a potential novel therapeutic target in SpA.

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SAT0357
LEVELS OF PERIPHERAL LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH ANKYLOSING SPONDYLITIS AND THEIR CHANGES AFTER RECEIVING IMMUNOREGULATORY COMBINATION THERAPIES
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Background: Ankylosing spondylitis is an immune-mediated inflammatory disease involving of the axial skeleton, joints, and enthesis1. Although the homeostatic balance of effector T cells (Teffs) and regulatory T cells (Tregs) is considered to play an important role in the pathogenesis of ankylosing spondylitis (AS)2, it is unclear whether the levels of peripheral blood lymphocyte subpopulations in patients with ankylosing spondylitis are abnormal or not.

Objectives: To explore the differences of lymphocyte subpopulations of peripheral blood (PB) between AS patients and healthy controls (HCs), and further evaluate the therapeutic effect of immunomodulatory drugs on the lymphocyte subpopulations.

Methods: Total 1141 patients with AS and 206 healthy individuals were enrolled in the study and donated their blood to measure the levels of T, B, NK, CD4+T, CD8+T, Th1, Th2, Th17 and Tregs by flow cytometry combined with standard absolute counting beads. And 456 patients received immunomodulatory combination treatments which includes low-dose interleukin-2, rapamycin, metformin, retinoic acid etc. and donated their PB after the therapies. Data were expressed as mean ± standard deviation to the distribution. Independent-samples T test and paired-samples T test were applied. P value <0.05 were considered statistically significant.

Results: Compared with HCs, AS patients had a lower absolute number of Tregs but higher numbers of peripheral T, B, CD4+T, CD8+T, Th1, Th2 and Th17 cells (P<0.05). Further, there was a significant increase in the percentage of B, CD4+T and the ratios of Teffs/Tregs such as Th1/Tregs, Th2/Tregs and Th17/Tregs compared with HCs (P<0.05)(Figure 1). Although, after receiving the immunomodulatory combination treatments, the absolute numbers of various peripheral lymphocyte subpopulations such as T, B, NK, CD4+T, CD8+T, Th1, Th17 and Tregs and the percentage of Tregs, Th1 and CD8+T significantly increased (P<0.05), the ratios of Th2/Tregs significantly decreased (P<0.05)(Figure 2), suggesting a rebalance of immune systems.

Conclusion: The insufficiency of Tregs may involve in pathogenesis of AS. Immunomodulatory combination therapies could promote the proliferation of Tregs as well as other lymphocytes to some degree, which may be a new target for AS treatment.

References: