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 HR-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 interesting target for upcoming treatment strategies in the near future.

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SAT0355

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 72 hours. 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 in 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- α .

Results: The level of OD of MOVAS cells treated with serum from AS patients (19.503 ± 6.422 , mean $\pm$ 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 (2.697 ± 1.46 ; 2.687 ± 1.753 ; 2.253 ± 1.128 ; 2.574 ± 1.142) was significantly higher than that from controls (1.396 ± 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 positive correlation between the mRNA level of WNT5a and RUNX2 ($\rho=0.705$, $p=0.002$, Spearman rank correlation coefficient) and the protein level of WNT5a and ALP, MMP-7 and TNF- α , MMP-7 and IL-17, MMP-7 and IL-23 ($\rho=0.601$, $p=0.039$; $\rho=0.769$, $p=0.026$; $\rho=0.828$, $p=0.011$; $\rho=0.777$, $p=0.003$), 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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SAT0356

THE ROLE OF IL-36 AS A POTENTIAL NOVEL THERAPEUTIC TARGET IN SPONDYLARTHROPATHY ASSOCIATED PATHOLOGY DUE TO ITS UPSTREAM INDUCTION OF IL-23/IL-17 PATHWAY CYTOKINES AND STROMAL ACTIVATION IN AN IN VITRO ENTHESITIS MODEL.

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Background: Enthesitis, defined as inflammation of anchorage points of tendons, ligaments and joint capsules to bones, is now understood to be the cardinal pathological lesion in spondyloarthropathies (SpA). Evidence from genetic studies, animal models, and therapeutic studies firmly implicates the IL-23/IL-17 axis in the pathogenesis of SpA. We have recently confirmed the presence of IL-23 myeloid cells and IL-17 producing T cells populations in the human enthesitis (McGonagle, ARD 2019). The upstream drivers of these key cytokines in the enthesitis is, however, not defined. Emerging evidence suggests that IL-36 may be critical in regulating the IL-23/IL-17 axis in various organs. Also IL-38 SNPs have been associated with SpA which is of interest given that IL-38 is an IL-36 antagonist. Further, IL-36 α is up-regulated in the joints of psoriatic arthritis patients and deficiency of the IL-36 receptor antagonist (DITRA) results in generalized pustular psoriasis with comorbid arthritis in at least third of patients. Thus, we hypothesised that IL-36 could be an upstream regulator of the IL23/17 axis at the enthesitis.

Objectives: To confirm the induction of IL-36 at the human enthesitis and to test the effect of IL-36 on resident innate and adaptive immune cell populations and enthesitis stromal cells.

Methods: Enthesial spinous processes from patients undergoing elective orthopaedic surgeries was obtained and mechanically digested. Peri-enthesial bone (PEB), and enthesial soft tissue (EST) digests were stimulated with fungal and bacterial adjuvants and IL-36 measured by ELISA. Disease relevant compounds such as methotrexate and PDE4i were assessed for their ability to attenuate IL-36 secretion. IHC was used to confirm the presence of IL-36R+ cells in the enthesitis. Digested PEB was stimulated with IL-36, and IL-6, IL-8, IL-23, and TNF-alpha were analysed by ELISA and Flow Cytometry. As the IL-36 cytokines require protease mediated post translational processing for full activation, these were measured in enthesitis digests. Enthesial fibroblasts were isolated and stimulated with IL-36 and ICAM-1 measured by Flow Cytometry and genes by qPCR.

Results: TLR ligands induced the production of IL-36 at the enthesitis. Further cell sorting, revealed CD11+ myeloid cells were the predominant enthesial producer of IL-36. Induced IL-36 could be significantly attenuated by PDE4i but not by methotrexate. IHC confirmed the presence of IL-36R+ in the enthesitis. Stimulation of enthesitis digest with IL-36 significantly upregulated the production of IL-6, IL-8, TNF- α , and IL-23. Stimulation of enthesitis fibroblasts with IL-36 upregulated surface ICAM-1 and secretion of IL-6, CCL2 and CCL20. Enthesitis digests showed high basal expression of IL-36 activating protease including cathepsin S and G.

Conclusion: IL-36 is inducible from enthesitis myeloid cells and IL-36R+ cells are present at the enthesitis. Enthesitis stimulation with IL-36 results in the upregulation