among ILCs. Multivariate linear regression and Receiver-Operating Characteris-
tic (ROC) Curve analysis was performed using the IBM SPSS Statistics software.
Different in vivo models were used to assess functional implications of ILCs at
various anatomical locations. Also, IL-36 SNPs have been associated with SpA
such as methotrexate and PDE4i were assessed for their ability to attenuate
the formation of 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 enthesis (McGonagle, ARD
2019). The upstream drivers of these key cytokines in the enthesis is, however, not
fully defined. Emerging evidence suggests that IL-36 may be critical in regulating the
enthesis stimulation with IL-36 results in the upregulation
of IL-36. Induced IL-36 could be significantly attenuated by PDE4i but not by meth-
otrexate. IHC confirmed the presence of IL-36R+ in the enthesis. Stimulation of
the enthesis digest with IL-36 significantly upregulated the production of IL-6, IL-8,
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-27) 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-23 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 ENTHESIS 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 cardiotic patho-
genesis lesion in spondyloarthopathies (SpA). Evidence from genetic studies, animal
models, and therapeutic studies firmly implicates the IL-23/IL-17 axis in the patho-
genesis of SpA. We have recently confirmed the presence of IL-23 myeloid cells and IL-17 producing T cells populations in the human enthesis (McGonagle, ARD
2019). The upstream drivers of these key cytokines in the enthesis is, however, not
defined. Emerging evidence suggests that IL-36 may be critical in regulating the
enthesis stimulation with IL-36 results in the upregulation
of IL-36. Induced IL-36 could be significantly attenuated by PDE4i but not by meth-
otrexate. IHC confirmed the presence of IL-36R+ in the enthesis. Stimulation of
the enthesis digest with IL-36 significantly upregulated the production of IL-6, IL-8,
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.
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SAT0355

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


Background: Vascular calcification is highly correlated with atherosclerosis. Ankylosing spondylitis (AS) is associated with a process of accelerated athero-
sclerosis. 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 Col-
collection, ATCC® CRL-7797™) 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 stim-
ulated 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-den-
sity lipoprotein receptor-related protein (LRP)5, LRP6, Dickkopf-related protein 1. Wnt3a, matrix metalloproteinase-7(MMP-7), beta-catenin for canonical Wnt sig-
naling; 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, L23 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 (2.687 ± 1.46; 2.687 ± 1.753; 2.253 ± 1.129; 1.574 ± 1.142) was significantly higher than that from controls (1.396 ± 0.587; 1.696 ± 0.637; 1.358 ± 0.473; 1.368 ± 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 (r=0.705, p=0.002, Spear-
man 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-27) 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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Sci en tifi c Abstracts

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 entheses. 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), 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 immunoregulatory 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 immunoregulatory 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 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 immunoregulatory 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. Immunoregulatory 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:**
