involvement we observed that gp130/sIL-6Rb, sIL-6Ra, IL-35, and TSLP serum levels were significant enhanced in MO-BD compared to M-BD subgroup.

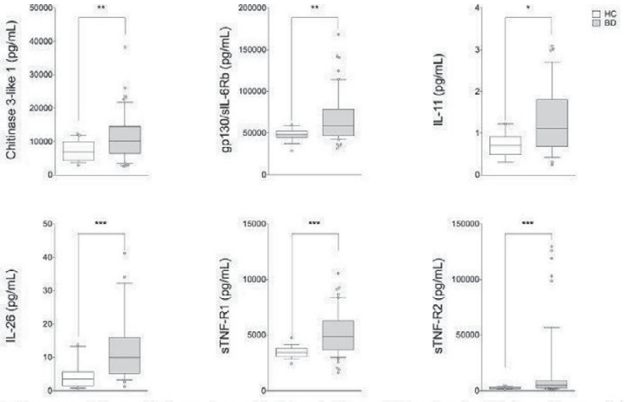


Fig.1. Serum cytokine profile in patients with Behçet's disease. BD patients (n=54) showed up-regulation of serum levels of Chitinase3-like1, gp130/sIL-6Rb, IL-11, IL-26, sTNF-R1 and sTNF-R2 compared with HC (n=19). Mann-Whitney U-test as well as Student's t-test were carried out to check for statistical significance between groups when required (***p<0.001, **p<0.01, *p<0.05). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. Abbreviations: HC, healthy controls; BD, Behçet's disease.

Conclusions: Our findings showed a signature of IL-6, TNF-α as well as of Th17 response in BD patients due to increased levels of gp130/sIL-6Rb, sTNF-R1, sTNF-R2, IL-26 respectively. This evidence could contribute to improve the knowledge regarding the role of these citokines in the induction of specific BD clinical features

References:

[1] Lopalco G, Lucherini OM, Vitale A, Talarico R, Lopalco A, Galeazzi M, et al. Putative Role of Serum Amyloid-A and Proinflammatory Cytokines as Biomarkers for Behcet's Disease. Medicine (Baltimore) (2015) 94(42):e1858.
[2] Lucherini OM, Lopalco G, Cantarini L, Vitale A, Rotondo C, Lopalco A, et al. Correlation between serum amyloid-A and serum levels of proinflammatory cytokines in patients with Behçet's disease. Pediatric Rheumatology Online Journal (2015) 13(Suppl 1):P6.
[3] Turan B, Pfister K, Diener PA, Hell M, Möller B, Boyvat A, et al. Soluble tumour necrosis factor receptors sTNFR1 and sTNFR2 are produced at sites of inflammation and are markers of arthritis activity in Behçet's disease. Scand J Rheumatol (2008) 37(2):135–41.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1935

AB0038 INCREASED INTERFERON-ALPHA PRODUCTION BY PLASMACYTOID DENDRITIC CELLS STIMULATED WITH A TLR-7 AGONIST IN SYSTEMIC LUPUS ERYTHEMATOSUS

G. Murayama [1,2], N. Furusawa [2], A. Chiba [2], K. Yamaji [1], N. Tamura [1], S. Miyake [2].
[1] Department of Internal Medicine and Rheumatology; [2] Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan

Background: Type I interferon (IFN) appears to contribute to the development of systemic lupus erythematosus (SLE). IFN-α production is known to be increased in peripheral blood mononuclear cells (PBMCs) from SLE patients. Although plasmacytoid dendritic cells (pDCs) is a major source of IFN-α, previous reports showed that IFN-α production by pDCs stimulated with a TLR-9 agonist was decreased in SLE compared to healthy controls (HC).
Objectives: We set out to investigate an other endosomal TLR-signaling pathway in SLE by using TLR-7 agonist stimulation.
Methods: Blood samples were obtained from 55 HC and 73 SLE patients, diagnosed according to the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus (2012). PBMC from SLE patients and HC were stimulated with a TLR-9 agonist, CpG-A oligodeoxynucleotides (CpG-A ODN)-2216, and a TLR-7 agonist, imiquimod. The proportion of pDCs producing IFN-α was investigated by intracellular cytokine staining and flowcytometory. PBMC were pretreated with IFN-α for 24 hours, and then IFN-α production by pDCs was assessed after imiquimod stimulation.
Results: As previously reported, the level of IFN-α production by pDCs stimulated with CpG-A ODN was reduced in SLE compared with HC. However, the proportion of IFN-α producing pDCs stimulated with imiquimod was significantly increased in SLE patients. The percentage of IFN-α producing pDCs stimulated with imiquimod was positively correlated with SLE disease activity index (SLEDAI) score, and that of pDCs stimulated with CpG-A ODN was negatively correlated with SLEDAI. The expression of TLR-7 on pDCs, but not TLR-9, was upregulated in SLE patients compared with HC. Furthermore, pretreatment with IFN-α increased IFN-α production by pDCs upon imiquimod stimulation.
Conclusions: IFN-α production by pDCs form SLE patients was increased when stimulated with a TLR-7 agonist, and this was accompanied with upregulated TLR-7 expression in these cells. In murine lupus-models, TLR7-deletion has been shown to reduce autoimmune disease. The enhanced TLR-7 signaling pathway in pDC may play an important role in lupus pathology.

References:

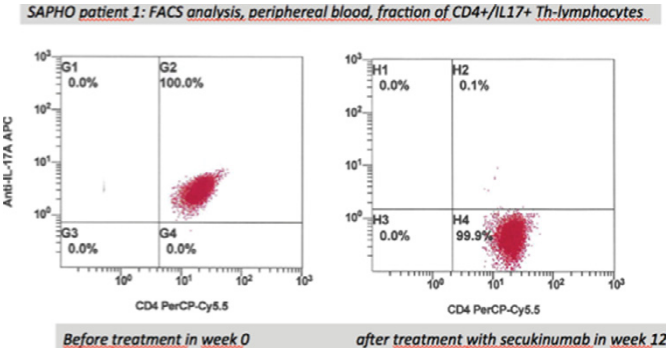
[1] Christensen SR, et al. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity. 2006; 25: 417–28.
[2] Sacre K, et al. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus. Arthritis Res Ther. 2012;R155.
[3] Fukui R, et al. Unc93B1 restricts systemic lethal inflammation by orchestrating Toll-like receptor 7 and 9 trafficking. Immunity 2011; 35: 69–81.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3074

AB0039 REDUCTION OF TH17+ LYMPHOCYTES IN PART OF SAPHO PATIENTS ON TREATMENT WITH SECUKINUMAB

G. Assmann [1], A. Tajali [2], S. Bamberger [1], M. Pfreundschuh [1], C. Schormann [1], F. Neumann [1]. *[1] Rheumatology and Oncology, University Medical School of Saarland, Homburg; [2] Rheumatology, Püttlingen Hospital, Püttlingen, Germany*

Background: The SAPHO syndrome has to be considered as a rare subtype of the disease entity of the seronegative spondylarthritis. The characteristic defining symptoms are synovitis, acne, palmoplantar pustulosis (PPP), and hyperostosis with osteitis. In general, most of SAPHO patients complete the diagnostic criteria for spondylarthritis and/or psoriatic arthritis. The etiology of SAPHO syndrome remains unclear so far, autoimmune dysregulations potentially triggered by bacterial infection with propionibacterium acnes has been discussed. Firinu D et. al (Ref.) has previously published data of higher Th17+ lymphocytes in the peripheral blood in SAPHO patients compared with psoriatic arthritis patients or healthy controls. Activation of the TH17 pathway leads to pro-inflammatory effects mediated by interleukin 17 with stimulation of osteoblast, macrophages, and fibroblasts with the consequences of secretion f pro-inflammatory cytokines such as interleukin 6 and 1, TNF alpha, and MMPs. The interleukin 17 blocking agent secukinumab has been introduced in the armentarium of antirheumatic drugs against seronegative spondylarthritis including psoriatic arthritis.
Objectives: To evaluate the count of Th17+ lymphocytes in patients with SAPHO syndrome and psoriatic arthritis before and under treatment with secukinumab.
Methods: Peripheral blood was derived from 4 patients with SAPHO syndrome and 4 patients with psoriatic arthritis, respectively before and under 12 week treatment with secukinumab 300mg (dosage: 4 times weekly, then monthly). All patients had received at least one conventional DMARDs and one TNF blocking agent in their medical history. All patients showed active disease with elevated scores of DAS28 and/or HAQ, for SAPHO patients the activity scores of osteitis (from 0 to 6) and PPP (0–6) were estimated by physician. The blood specimen were separated in EDTA containing tubes to separate lymphocytes, which were measured using FACS analysis to evaluate the fraction of Th 17+/CD4+ lymphocytes. The Ethics Committee of Saarland has proven the study, all patients gave their consent to take part in the study.
Results: The Th17+lymphocytes were not detectable in 4 patients with psoriatic arthritis and 2 of 4 SAPHO patients before and under 12 week treatment with secukinumab. In 2 of 4 SAPHO patients the fractions of Th17+ lymphocytes were prominent prior to secukinumab application; after treatment duration of 12 weeks one of both developed a depletion of Th17+ cells (figure), the other SAPHO patient a Th17+ cell reduction. Only the two SAPHO patients with diminishing Th17+ lymphocytes have developed treatment response evaluated by reduction of HAQ score (from 1.75 to 1.25), osteitis score (4.5 to 3.0), and PPP score (5.0 to 4.0). Three of 4 psoriatic arthritis patients showed reduced diseases activity under treatment with secukinumab (DAS28 score from 4.22 to 3.45, HAQ 2.25 to 1.5).



Conclusions: The measurement of Th17+lymphocytes in the peripheral blood of SAPHO patients could be suggested for further evaluation as possible predictor of treatment response by secukinumab.

References:

[1] Firinu D, Barca MP, Lorrai MM, Perra S, Cabras S, Muggianu E, Di Martino ML,

Manconi PE, Del Giacco SR. TH17 cells are increased in the peripheral blood of patients with SAPHO syndrome. Autoimmunity. 2014 Sep;47(6):389–94. doi: 10.3109/08916934.2014.906582. Epub 2014 Apr 10.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4360

AB0040 IMMUNE MODULATORY EFFECTS OF MESENCHYMAL STEM CELL TO MONONUCLEAR CELLS FROM PATIENTS WITH ACTIVE ADULT ONSET STILL'S DISEASE

I.W. Sohn[1], H.-J. Jeong[2], G.Y. Ahn[1], M.J. Kim[1], H. Kim[1], S. Kang[1], D.H. Yoo[1]. *[1]Rheumatology, Hanyang University Hospital for Rheumtic diseases, Seoul; [2]Rheumatology, Keimyung University Dongsan Medical Center, Daegu, Korea, Republic Of*

Background: Adult onset Still's disease (AOSD) is an inflammatory disorder of unknown etiology, which is accompanied by increased levels of serum pro-inflammatory cytokine. Mesenchymal stem cells (MSCs) have immunomodulatory capacities and might be a promising therapeutic option in the treatment of refractory autoimmune diseases. Both cell-to-cell contact and the release of soluble factors mediate immune modulatory functions of MSCs.
Objectives: We aimed to determine if MSCs could modulate serum cytokine level in patients with active untreated AOSD, either through paracrine secretion or via direct contacts with the MSCs.
Methods: Human peripheral blood mononuclear cells (hPBMCs) from 6 patients with active AOSD were co-cultured for 72 hours with human MSCs (hMSCs at a ratio of 10 to 1). We compared the cytokine levels before and after direct or indirect (transwell cultures) exposition to activated mononuclear cells (LPS, 10ng/ml) or T cell-inducing conditions (anti-CD3 [5 μg/ml], anti-CD28 [5 μg/ml], recombinant human IL-2 [5 ng/ml]). Cytokine levels were detected by multiplex cytokine detection kit by flow cytometry, or ELISA with culture supernatant. In vitro platform for studying the effects of MSCs on individual cytokines, the Wilcoxon signed-rank test was employed for comparison of serum cytokine levels.
Results: Treatment of mononuclear cells with hMSCs resulted in significant reduction of mean TNF-α level (mean 463.4 pg/ml vs 137.8 pg/ml, $p<0.05$) and IL-1 β (mean 1887.1 pg/ml vs 1127.9 pg/ml, $p<0.05$). When the hMSCs were present during the T-cell differentiation, there was a significant decrease in the mean secreted TNF-α (mean 10953.5 pg/ml vs 454.9 pg/ml, $p<0.05$), IFN-γ (mean 14301.0 pg/ml vs 5090.4 pg/ml, $p<0.05$) and sIL-2 receptor (mean 3550.8 pg/ml vs 2506.4 pg/ml, $p<0.05$). On the contrary, level of TGF-β was significantly increased (mean 4088.8 pg/ml vs 5104.8 pg/ml, $p<0.05$). But, there was a significant increase in the amount of IL-6 (mean 2215.5 pg/ml vs 25130.6 pg/ml, $p<0.05$) and IL-17 α (mean 1357.0 pg/ml vs 2453.6 pg/ml, $p<0.05$). Two chamber experiments also showed similar pattern of cytokine modulation.
Conclusions: This preliminary experiment demonstrated that MSCs can modulate cyokine profiles of AOSD mononuclear cells by decreasing pro-inflammatory cytokines, and increasing anti-inflammatory cytokine such as TGF-β. However, up-regulation of IL-6 and IL-17 might be a hurdle to overvome in the clinical application of MSCs in AOSD patients.
References:
[1] Serum cytokine profiles in patients with Adult onset Still's disease. J Rheumatol. 2003 Nov;30(11):2422–7.
[2] Human mesenchymal stem cells modulated allogeneic immune cell responses. Blood. 2005 Feb 15;105(4):1815–22. Epub 2004 Oct 19.
Acknowledgements: none.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5719

AB0041 LARGE VESSEL VASCULITIS INDUCED BY CANDIDA ALBICAN WATER-SOLUBLE-FRACTION (CAWS) IN THE C57BL/6J MOUSE MODEL IS ASSOCIATED WITH OVEREXPRESSION OF IL-6, TNF-α, AND IL-10 WITH MODEST CHANGE IN SOCS-1

J.M. Springer[1], M. Zhang[2], D. Smith[1], N. Miura[3], N. Ohno[3], O. Tawfik[4], M. Maz[1], K. Dileepan[1]. *[1]Allergy, Immunology and Rheumatology; [2]Department of Orthopedics, Kansas University Medical Center, Kansas City, United States; [3]Department of Pharmacy and Life Sciences, Tokyo University, Hachioji, Japan; [4]Department of Pathology and Laboratory Medicine, Kansas University Medical Center, Kansas City, United States*

Background: We have previously demonstrated that mast cell degranulation acutely downregulates lipopolysaccharide induced aortic expression and serum levels of IL-6 in vivo. This is accompanied by aortic upregulation of suppressor of cytokine signaling-1 (SOCS-1) gene expression[1]. This effect is not seen in histamine H1 receptor-knockout mice suggesting that mast cell-derived histamine is a key mediator involved in IL-6 homoeostasis[2]. Mice injected with *Candida albican* water-soluble-fraction (CAWS) have been shown to develop coronary and aortic vasculitis[3]. Our long-term objective is to determine the pathogenic mechanism of large vessel vasculitis (LVV).
Objectives: The aim of this pilot study was to replicate and develop a working mouse model to determine the regulatory role of mast cells in LVV.
Methods: Eight to ten weeks-old male C57Bl6/J mice were randomly distributed into two groups [CAWS, N=8; and Control, N=8] and were injected i.p. daily for 5 days with either CAWS in normal saline (2 mg/day/mouse) or normal saline alone (controls). All mice were sacrificed 30 days after the 5th injection. We examined serum levels of IL-6 and TNF-α, as well as aortic tissue expressions of IL-6, TNF-a, IL-10 and SOCS-1 mRNA. Heart and aortic sections were evaluated for inflammation and mast cells after staining with H & E and toluidine blue, respectively.
Results: Treatment of mice with CAWS for 5-consecutive days led to overexpression of IL-6, TNF-a and IL-10 genes in the aortic tissue with modest upregulation of SOCS-1. At the root of the aorta, all animals in the CAWS group had intense inflammatory infiltrates composed of mixed acute and chronic inflammatory cells. There is also evidence of vasculitis in the coronary arteries. In contrast, none of the control mice had any evidence of aortic inflammation or vasculitis. Serum IL-6 concentrations were below detectable levels in both controls and CAWS-treated mice whereas TNF-a levels were elevated in 3 out of 8 mice in the CAWS group. There were no signs or increased presence of intact or degranulating mast cells in the area of inflammation.
Conclusions: These results suggest that CAWS-induced LVV involves acute and chronic inflammatory response and vascular tissue expression of both pro- and anti-inflammatory cytokines and SOC-1. Detailed kinetic studies are warranted to determine the optimum windows of peak inflammatory response and the expression of these genes to understand the pathobiology of CAWS-induced LVV.
References:
[1] Springer J, Raveendan V, Smith D, et al. [abstract] Ann Rheum Dis 2014; 73:112–113.
[2] Raveendran V, Springer J, Smith D, et al. [abstract]. Arthritis Rheumatol 2015; 67(suppl 10).
[3] Miura N, Shingo Y, Adachi Y, et al. Immunopharmacol Immunotoxicol 2004; 26(4):527–43.
Acknowledgements: Supported by Basic Science Development Award from Department of Medicine at the University of Kansas.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4884

AB0042 HIGH EXPRESSION OF S100 CALGRANULINS GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH TAKAYASU ARTERITIS

J. Kabeerdoss, R. Goel, H. Mohan, D. Danda. *Department of Rheumatology, CHRistian Medical College, Vellore, India*

Background: Takayasu arteritis (TA) is inflammatory disorder that affects aorta and its branches. Toll-like receptors (TLR) 1 to 4 are highly expressed in aorta (1). Activation of TLR4 causes transmural arteritis in human temporal artery–SCID chimera model (2). Ligand responsible for TLR4 activation is not known in TA.
Objectives: Aim of the study is to examine the expression of TLR4 and its endogenous ligands in peripheral blood mononuclear cells (PBMCs) of patient with TA.
Methods: RNA from PBMCs of 24 TA patients and 19 sex and age matched healthy controls were extracted. The mRNA expression of various endogenous TLR4 ligands, TLR4, RAGE, interleukin-6 (IL-6) and IL-8 were quantified in real time PCR using specific primers and SYBR Green qPCR master mix. Serum S100A8/A9 and S100A12 levels were measured using commercial ELISA kits. S100A8/A9 and S100A12 were measured in cell culture supernatant of un-stimulated and lipopolysaccharides (LPS) stimulated PBMCs, cultured for 4 hours. t-test was used to compare between the groups. $P<0.05$ was considered as statistically significant.
Results: The mRNA of S100A8, S100A9, S100A12 and TLR4 were highly expressed in TA as compared to healthy controls, while RAGE, HSP70 and IL-6 had lower expression in TA. No difference in serum levels of S100A8/A9 and S100A12 was noted between TA and healthy controls. LPS induced high secretion of both S100A8/A9 and S100A12 levels in both TA and healthy controls (Figure-1). However, the stimulatory response in healthy controls [2.88 (1.7–3.53) fold] was significantly higher as compared to TA [1.345 (1–1.82) fold; $p<0.05$] as measured by delta S100A12 (LPS/unstimulated control). Numerically delta S100A8/A9 was also higher in healthy controls [2.04 (1.7–5.6) fold] as compared to TA [1.38 (1.09–3.6) fold; p=0.129].

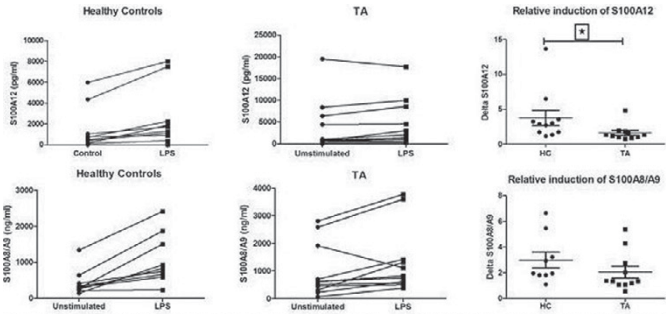


Figure -1. S100A8/A9 and S100A12 secretion by PBMCs cultured with and without LPS (100ng/ml) in RPMI medium for 4 hours for TA (n=10) and Healthy controls (n=10). Each circle represents each subjects and connecting line represents their corresponding S100A8/A9 and S10012 secretion levels in unstimulated control (without LPS) and LPS treatment on PBMCs. *=p<0.05.