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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

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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.
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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

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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.
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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

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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].

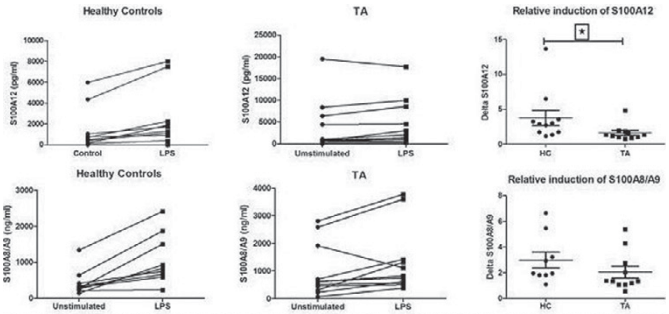

Figure -1. S100A8/A9 and S100A12 secretion by PBMCs cultured with and without LPS (100ng/ml) in RPMI medium for 4 hours for TA (n=10) and Healthy controls (n=10). Each circle represents each subjects and connecting line represents their corresponding S100A8/A9 and S10012 secretion levels in unstimulated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