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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 IL-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 cytokine 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 overcome in the clinical application of MSCs in AOSD patients.

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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 expression¹. 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 homeostasis². Mice injected with *Candida albican* water-soluble-fraction (CAWS) have been shown to develop coronary and aortic vasculitis³. 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 C57BL/6J 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-α, 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-α 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-α 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 SOCS-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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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 S100A12 secretion levels in unstimulated control (without LPS) and LPS treatment on PBMCs. *p<0.05.

Conclusions: mRNA expression of S100A8, S100A9, S100A12 and TLR4 in PBMCs of TA patients were higher as compared to healthy controls. Despite this, S100A12 secretion was lower in TA as compared to healthy controls upon LPS challenge.

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AB0043 TLR4 INHIBITION REDUCES MOVEMENT-INDUCED NOCICEPTION AND ATF-3 EXPRESSION IN EXPERIMENTAL OSTEOARTHRITIS

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Background: Toll-like receptor 4 (TLR4) is a pattern recognition receptor involved in the initiation of inflammatory responses to control pathogen infections, but is also a “danger-sensing” receptor that recognizes host-derived endogenous molecules called damage-associated molecular patterns (DAMPs). The role of TLR4 in rheumatic diseases is becoming evident, as well as its potential role as a therapeutic option.

Objectives: The aim of this study was to evaluate the effect, both on articular histopathology and pain behaviour, of a TLR4 antagonist (TLR4-A1) on an experimental model of OA. The effect of the TLR4-A1 on the activating transcription factor-3 (ATF-3) signalling pathway was also assessed.

Methods: OA was induced in adult Wistar rats through an intra-articular injection of 2mg of sodium mono-iodoacetate (MIA) into the left knee. Control animals received a similar injection with saline. TLR4-A1 (10 mg/kg), synthesized by Dr. Quesada from a compound previously described by the Peri Laboratory (Piazza et al., 2009), was intraperitoneally administered, daily, from days 14 to 28 after OA induction. Under the same procedure a control group of animals received the vehicle. Movement- and loading- induced nociception was evaluated in all animals, by the Knee-Bend and CatWalk tests, before and after TLR4-A1 or vehicle administration, at several time-points. Animals were sacrificed 28 days after OA induction. L3-L5 Dorsal Root Ganglia (DRG) were used for immunohistochemistry for TLR4 and ATF-3, spinal cords were immunoreacted for TLR4 and knee joints were processed for histopathological evaluation.

Results: Antagonism by TLR4-A1 significantly reduced the nociceptive behavior of OA animals both in the Knee-Bend and Catwalk tests. The effect was immediately observed 1 day after TLR4-A1 administration, but became more evident 4 days later, maintaining thereafter. No improvement in the cartilage histology was observed. The increased ATF-3 expression observed in DRG of OA animals was significantly reduced by TLR4-A1. On the contrary, TLR4 expression slightly increased after antagonist administration both at DRG and superficial dorsal horn levels.

Conclusions: Chronic treatment with TLR4-A1 showed an antinociceptive effect on OA animals, not related to articular histopathological improvement, possibly through an ATF-3 dependent mechanism.

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AB0044 THE INHIBITORY EFFECTS OF TACROLIMUS ON GLIOSTATIN PRODUCTION IN RA SYNOVIOCYTES

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Background: Gliostatin (GLS) is known to have angiogenic and arthritogenic activities¹. We also reported a decrease in serum GLS levels in RA patient responders treated with conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs)². Tacrolimus (TAC), an orally available calcineurin inhibitor, is a potent immunosuppressant. The efficacy of TAC monotherapy and the combination therapy with TAC and methotrexate in the treatment of RA has been previously described³. Although the principal TAC action is thought to be the inhibition of T cell activation, its beneficial effect on RA synoviocytes has not been yet elucidated.

Objectives: To determine the inhibitory effects of TAC on GLS production in RA, we investigated the modulation of serum GLS by TAC therapy and the effect of TAC on the production of GLS in cultured fibroblast-like synoviocytes (FLSs).

Methods: Serum samples were collected from eleven RA patients with active disease at baseline and after 12 weeks of TAC treatment. These RA patients had a history of unsatisfied response with at least one csDMARD or biological DMARD. Serum concentrations of GLS and matrix metalloproteinase (MMP)-3 were measured by enzyme immunoassay (EIA). Synovial specimens were obtained from RA patients at the time of total knee arthroplasty. FLSs were cultured and stimulated by TNF alpha with or without TAC. The expression levels of GLS were determined using RT-PCR and EIA. MMP-3 protein in conditioned media was measured by using EIA.

Results: Six patients fulfilled good and moderate responder and the other five patients fulfilled no responder with EULAR response criteria. DAS28, serum GLS, CRP and MMP-3 were significantly down-regulated in TAC responders. In RA FLSs, GLS mRNA and protein were significantly induced after treatment with TNF alpha alone (GLS mRNA 21.9-fold, protein 1.5-fold compared to control). These inductions were suppressed by TAC in a dose-dependent manner. MMP-3 protein was induced by TNF alpha and was similarly suppressed by TAC in a dose-dependent manner (MMP-3 protein 2.9-fold compared to control).

Conclusions: This study is the first to demonstrate that TAC down-regulates GLS mRNA and protein in FLSs treated by TNF alpha. Our data suggests that the beneficial effect of TAC in RA might be due to, at least in part, to anti-angiogenic and anti-arthritogenic activity following the down-regulation of GLS.

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AB0045 IL-17 INDUCED GLUCOCORTICOID INSENSITIVITY MIGHT BE DEPENDENT ON THE REDUCED 11BETA-HSD1 ENZYME ACTIVITY

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Background: It has been demonstrated that IL-17A is able to induce GC insensitivity. Although several studies have reported the role of IL-17 in GC insensitivity the mechanism underlying remains still largely unclear.

Objectives: To understand the effects of interleukin-17 (IL-17) on the enzyme 11β-hydroxysteroid dehydrogenases (11β-HSDs) in the two main classes of monocytes, CD14 and CD16.

Methods: Peripheral Blood Mononuclear Cells (PBMCs) were isolated from 5 healthy donors and were sorted into CD14 and CD16 subpopulations using cell sorting. Effect of IL-17 on 11β-HSD1 enzyme activity was measured in terms of conversion of cortisone to cortisol in sorted and unsorted monocytes using Homogeneous Time-Resolved Fluorescence (HTRF). The direct involvement of 11β-HSD1 in the conversion of cortisone to cortisol was confirmed using carbenoxolone, an inhibitor of 11β-HSD1.

Results: Monocytes showed a concentration-dependent decrease in the 11β-HSD1 enzyme activity when incubated with increasing concentrations of IL-17. CD14 and CD16 cells stimulated similarly with IL-17 showed a significant