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.

References:

- [1] Pryschep O, Ma-Krupa W, Younge BR, et al (2008) Vessel-specific Toll-like receptor profiles in human medium and large arteries. *Circulation* 118:1276–1284.
- [2] Deng J, Ma-Krupa W, Gewirtz AT, et al (2009) TLR4 and TLR5 induce distinct types of vasculitis. *Circ Res* 104:488–495.

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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.

References:

- [1] Piazza M, Rossini C, Fiorentina SD, Pozzi C, Cornelli F, Bettoni I, Fusi P, Costa B and Peri F. Glycolipids and Benzylammonium Lipids as Novel Antisepsis Agents: Synthesis and Biological Characterization. *J Med Chem* 2009, 52, 1209–1213.

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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 synovocytes 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 synovocytes (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.

References:

- [1] Asai K, et al. High concentration of immunoreactive gliostatin/ platelet-derived endothelial cell growth factor in synovial fluid and serum of rheumatoid arthritis. *Clin Chim Acta* 1993;218:1–4.
- [2] Kusabe T, et al. The inhibitory effect of disease-modifying anti-rheumatic drugs and steroids on gliostatin/platelet-derived endothelial cell growth factor production in human fibroblast-like synovocytes. *Rheumatol Int* 2005;25:625–630.
- [3] Kremer JM, et al. Tacrolimus in rheumatoid arthritis patients receiving concomitant methotrexate: a six-month, open-label study. *Arthritis Rheum* 2003;48:2763–2768.

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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