AB0078

SYSTEMIC AND LOCAL IL-17A AND MIR-223 LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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Background: IL-17 is a proinflammatory cytokine, which overproduction promotes the autoimmune reaction in rheumatoid arthritis (RA). Recent studies have shown that IL-17 production in lymphocytes or its function could be regulated by miR-223 by targeting Roquin ubiquitin ligase or its receptors^{1–4}.

Objectives: To examine a possible correlation between the expression levels of miR-223 and IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods: Expression levels of miR-223 were determined in matched PB and SF samples of RA patients by relative quantitation method 2^{-ΔΔCt}. As reference control for normalisation RNU6B gene was used. Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to healthy control (HCs) as well as within the RA group.

Results: 58.73% of the RA patients showed overexpression of miR-223 in PB when compared to HCs (p=0.008) with AUC=0.673 (95 CI: 0.562/0.784), with 71.4% sensitivity and 46.9% specificity (p=0.006). miR-223 was overexpressed in 79.17% of RA SF (p=1.64 × 10⁻³) when compared to HCs SF with AUC=0.841 (95 CI: 0.724/0.958) with 87.5% sensitivity and 72.7% specificity (p=4.6 × 10⁻⁴). Within the RA group, SF miR-223 was underexpressed in 58.7% of the patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, p=0.012).

Conclusions: The difference between the systemic and local levels of miR-223 and IL17A in RA patients shows that the inflammatory disease process leads to their altered expression with a possible role of both molecules in the disease pathogenesis. The opposite changes in their systemic and local levels confirm the data about the possible role of miR-223 in regulating IL-17 function.

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Acknowledgements: The study was supported by Grant 14D/2012 and Grant 61/2015 funded by Medical University-Sofia, Bulgaria

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5803

AB0079

ALFACALCIDOL SUPPLEMENTATION MODULATES CYTOKINE PRODUCTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS CULTURE OF PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS

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Background: Hormone D and its analogues display immunomodulatory activities that provide a beneficial effect in immunoinflammatory diseases. However, whether this hormone has an additive immunosuppressive effect when it is used with corticosteroids has not been investigated, although these agents are commonly used together.

Objectives: Testing the immunomodulatory *in vitro* effects of vitamin D analogue, alfacalcidol, in peripheral blood mononuclear cells (PBMC) cultures of patients with active rheumatoid arthritis (RA).

Methods: Sixteen patients with active RA were enrolled in the study. Patients PBMCs were isolated, stimulated with PMA/Ionomycin and cultivated for 48 hours at 37°C, 5% CO₂ in cell cultures medium with or without supplementation. *In vitro* effects of supplementation with alfacalcidol (concentration

10 nM), calcitriol (concentration 10 nM), methylprednisolone (concentration 400 nM) and cotreatment with alfacalcidol/methylprednisolone on cytokine production were studied. Stimulated production of cytokines IL-6, IL-17, IL-21, TNF-α, IL-4, IL-10, TGF-β and IFN-γ were determined in cell culture supernatant by standard ELISA method.

Results: *In vitro* alfacalcidol supplementation reduces the production of proinflammatory cytokines IL-17 (p=0.001), IL-21 (p=0.001), TNF-α (p=0.002) and IL-6 (p=0.4), and induce more intense anti-inflammatory cytokine production IL-4 (p<0.0001), IFN-γ (p=0.05), TGF-β (p<0.0001) and IL-10 (p=0.09), in PBMC cultures of patients with active RA. There was no significant difference between the effects of alfacalcidol and calcitriol, except decreased TGF-β production (p=0.535) after calcitriol supplementation. Methylprednisolone supplementation leads to a significant reduction of the IL-6 (p=0.002), IL-17 (p=0.0001), TNF-α (p=0.0001) and IL-4 (p=0.017) production, while increased production of IL-21, IL-10, IFN-γ didn't reach statistical significance. Cotreatment with alfacalcidol and methylprednisolone leads to additional, more significant reduction of IL-17 (p=0.0001) and TNF-α (p<0.0001) in PBMCs of patients with active disease.

Conclusions: Alfacalcidol, *in vitro*, showed a significant immunomodulatory effect through the specific inhibition of Th1- cytokine production, while Th2 cell response was enhanced – “Th2 switch”. Our results demonstrate that alfacalcidol has significant additive effects on glucocorticoid-mediated inhibition of Th1 cytokine production when combined with methylprednisolone. These findings demonstrate the potential use of alfacalcidol as an immunosuppressive agent when combined with corticosteroids in Th1, but not Th2, immune response.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7550

AB0080

DIFFERENTIAL EFFECTS OF TR14 VERSUS DICLOFENAC ON PRO-RESOLVING LIPID MEDIATORS REVEALED BY RNASEQ

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Background: Anti-inflammatory agents are used widely in treating numerous pain and inflammatory conditions. With a focus on the specialised pro-resolving mediators (SPM) pathways in cutaneous wound repair in mice, the therapeutic activities of Tr14 (Traumeel), a multicomponent/multitarget natural product, and diclofenac (NSAID), a non-selective cyclooxygenase (COX) inhibitor were compared. COX inhibitors can block the synthesis of prostaglandins and thromboxanes from arachidonic acid, and can have important effects on the synthesis of resolvins and protectins produced by similar enzymes from eicosapentanoic and docosahexanoic acid substrates. Differential effects were identified via transcriptome analysis (RNAseq).

Objectives: To compare the transcriptomic changes after administration of Tr14 or diclofenac in a mouse cutaneous wound healing model, with particular emphasis on the SPM pathways, which include resolvins and protectins.

Methods: After abrasive wounding, the wounds were treated with topical Tr14 (34 mg/ml) in combination with subcutaneous Tr14 injections (9.5 mg/ml), or with subcutaneous Tr14 injections only, or topical diclofenac at clinically relevant doses (2 mg/ml). Skin samples were analysed for RNA transcript profiling by RNAseq at specific times (12 hour, 24 hour, 36 hour, 72 hour, 96 hour, 120 hour, 192 hour) after injury. Differentially expressed genes (DEGs) were computed at each time point between diclofenac vs control or Tr14 vs control, using EdgeR.

Results: At early time points (12–36 hour), Tr14-treated wounds, and to a lesser extent diclofenac-treated wounds, showed marked induction of 3 lipoxigenase

enzyme mRNAs involved in SPM synthesis. Even more striking was a pronounced effect of Tr14 at 12–36 hours on Fpr1 and Fpr2 mRNAs, which are the transmembrane receptors for the SPM lipid mediators. Consistent with elevated levels of enzymes regulating SPM synthesis, and SPR receptors, there was a noticeable decrease in the mRNA levels of the p65/RelA subunit of NFκB at 72–96 hours. NFκB is a critical transcription factor in inflammation, regulating numerous cytokines and chemokines.

Conclusions: Tr14 and diclofenac had very different effects on the SPM synthetic pathway after cutaneous wounding. Tr14 stimulated mRNA levels of several key regulators of SPM synthesis, and had a marked effect on the mRNA levels of the SPM receptors. Tr14, not diclofenac, suppressed mRNA levels for NFκB subunit p65/RelA, which may explain some of the anti-inflammatory and proresolving properties of Tr14.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3789

AB0081 **LOW LIPOCALIN-2 IN SYSTEMIC LUPUS ERYTHEMATOSUS PREGNANCIES- A POSSIBLE MECHANISM FOR LOSS OF TOLERANCE**

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Background: Lipocalin-2 (LCN2) has become increasingly relevant as a potential clinical biomarker of rheumatic diseases.¹ The biological role of LCN2 in the adaptive immunity is less understood. It has been shown that LCN2 can induce immune tolerance by upregulation of human leukocyte antigen G (HLA-G) expression and by expansion of T-regulatory cells.² LCN2-deficient mice have been found to be more susceptible to induction of autoimmunity.³ Systemic lupus erythematosus (SLE) is a disease associated with loss of tolerance. Pregnancy complications seen in SLE are also regarded as a consequence of immune dysregulation. LCN2 might therefore play a role as an immune modulator in SLE-pregnancies.

Objectives: The study objective was to obtain a better understanding of immune regulation in pregnant women with SLE. In this study, we analysed serum LCN2 and clinical parameters in women with RA, SLE and healthy controls during pregnancy and postpartum.

Methods: The Norwegian National Advisory Unit on Pregnancy and Rheumatic Diseases collects serum samples in a biobank from women with inflammatory rheumatic diseases. Samples were obtained before pregnancy, in each trimester and 6 weeks, 6 months and 12 months postpartum from pregnant women with SLE (n=28), RA (n=34) and healthy pregnant controls (n=19). A sandwich ELISA was used to measure LCN2 in the serum samples. The biobank database was linked to RevNatus, a Norwegian quality registry collecting comprehensive clinical data about these women.

Results: Our cohort of pregnant women with SLE and RA had low disease activity throughout pregnancy and 67%–95% used medication (table 1). LCN2 levels in serum samples from women with SLE were found significantly lower compared to samples from women with RA and healthy controls at all time-points (p<0.05) (graph).

Abstract AB0081 – Table 1. Disease activity and medication

	SLE patients			RA patients		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
Disease activity						
LAI-P Mean (range) [SD]	0,04 (0–0,25) [0,08]	0,02 (0–0,16) [0,05]	0,005 (0–0,08) [0,02]			
DAS28 Mean (range) [SD]				2,34 (1,79–3,91) [0,76]	2,57 (1,42–5,02) [1,06]	2,57 (1,79–4,90) [0,94]
Medication						
Prednisolone, receiving (%) (dose range [mg])	17 (2,5–7,5)	48 (2,5–7,5)	48 (2,5–10)	28 (2,5–10)	33 (2,5–15)	38 (2,5–12,5)
Other immunosuppressive receiving (>1*) (%)	96 (33)	91 (39)	95 (43)	67 (11)	76 (5)	80 (10)

*>1: more than one of the following: SLE: hydroxychloroquine; azathioprine, RA: sulfasalazine, methotrexate, hydroxychloroquine, TNF inhibitors

Abstract AB0081 – Figure 1

Conclusions: Pregnant women with SLE had lower levels of LCN2 compared to pregnant women with RA and healthy controls. Our cohort of women had well controlled disease, making it likely that our findings represent inherent biological differences rather than effects of disease activity. Low LCN2 levels can be a possible mechanism for loss of tolerance seen in SLE patients during pregnancy.

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Acknowledgements: This study is supported by grants from St. Olavs Hospital and Norsk Revmatikerforbund.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3637

AB0082 **CLINICALLY RELEVANT DISCREPANCIES BETWEEN DIFFERENT RHEUMATOID FACTOR ASSAYS**

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Background: Accurate measurements of rheumatoid factors (RFs), autoantibodies binding IgG, are important for diagnosing rheumatoid arthritis (RA) and for predicting disease course. Worldwide, various RF assays are being used that differ in technique and target antigens.

Objectives: To study whether assay choice leads to clinically important discrepancies in RF status and level.

Methods: RF measurements using 4 commercial RF assays were compared in 32 RF+ samples. Using ELISAs, the influence of the target antigen source—human IgG (hIgG) versus rabbit IgG (rIgG)—on measured RF levels was investigated in arthralgia patients and RA patients.

Results: Substantial discrepancies were found between RF levels measured in the four commercial assays. 6 samples (19%) with RF levels below or slightly above the cut-off in an rIgG-based assay were RF+ in three assays using hIgG as the target antigen, some with very high levels. Direct ELISA comparisons of RF reactivity against hIgG and rIgG estimated that among 173 ACPA+ arthralgia patients, originally RF negative in rIgG-based assays, up to 10% were single positive against hIgG. Monoclonal RFs binding to hIgG and rIgG or hIgG only supported these findings. In a cohort of 69 early RA patients, virtually all RF responses reacted with both targets, although levels were still variable.

Conclusions: The use of RF assays that differ in technique and target antigen, together with the different specificities of RF responses, leads to discrepancies in RF status and levels. This has important consequences for patient care if RA diagnosis and disease progression assessments are based on RF test results.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.1477