Objectives To investigate the role of icIL-1Ra1 in a mouse model of psoriasis-like skin inflammation induced by Aldara cream, an FDA-approved drug with the active ingredient imiquimod, a toll-like receptor 7 agonist.

Methods We generated a mouse line specifically lacking icIL-1Ra1, in which expression of the other three IL-1Ra isoforms was not affected. Psoriasis-like skin inflammation was induced on mouse ears by the topical application of Aldara cream. Skin inflammation was assessed using a caliper to measure ear thickness. Ear mRNA levels of cytokines were measured by real-time PCR. An intracellular or extracellular role of icIL-1Ra1 was investigated *ex vivo* on ear biopsies and *in vitro* in primary keratinocytes by real-time PCR or ELISA.

Results Naïve icIL-1Ra1 deficient mice exhibited a normal phenotype. However icIL-1Ra1 deficiency resulted in an enhanced severity of Aldara cream-induced skin inflammation as demonstrated by increased ear thickness. In addition, mRNA levels of several pro-inflammatory cytokines were significantly increased in icIL-1Ra1 deficient mice as compared to wild-type (WT) littermates. By immunofluorescence IL-1Ra was detected only in keratinocytes of naïve and Aldara-treated WT mice, but totally absent in icIL-1Ra1 deficient mice. Using ear biopsies and keratinocytes from WT mice, we observed that Aldara cream led to the release of both icIL-1Ra1 and the alarmin IL-1 α , accompanied by caspase 1/11-independent cell lysis. Injection of neutralizing anti-IL-1 α antibodies into Aldara-treated icIL-1Ra1 deficient mice significantly reduced the severity of skin inflammation.

Conclusions These data suggest that icIL-1Ra1 is stocked in keratinocytes in order to immediately counteract the inflammatory effects of IL-1 α that is released upon cell lysis, thus acting as an intrinsic alarmin inhibitor.

Disclosure of Interest None declared.

P099

COMPARISON OF IL-17A AND IL-17F EFFECTS ON DIFFERENT CELL TYPES

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10.1136/annrheumdis-2018-EWRR2019.87

Career situation of first and presenting author Post-doctoral fellow.

Introduction IL-17A has effects on a growing number of cell types, including synoviocytes, skin fibroblasts or endothelial cells. IL-17A is now a therapeutic target in several inflammatory diseases, including psoriasis, PsA and SPA. IL-17F is the closest member of IL-17A in the IL-17 family and it shares IL-17A biological activities. It is now of interest to target both IL-17A and F. Furthermore, IL-17A and IL-17F can synergize with other cytokines such as TNF or IL-1.

Objectives The aim of this study is to compare in the same experiments, the effect of IL-17A and IL-17F, in combination or not with TNF and IL-1 β , on pro-inflammatory cytokine production (IL-6 and IL-8) first by RA synoviocytes, then extended to other cell types.

Methods Cells (RA synoviocytes, psoriatic skin fibroblasts, human umbilical vein endothelial cells, myoblasts and hepatocytes) were cultured in presence or not of: IL-17A alone, IL-17F alone, TNF alone, IL-1 β alone or combinations, IL-17A/TNF, IL-17A/IL-1 β , IL-17A/TNF/IL-1 β , IL-17F/TNF, IL-17F/IL-

1 β IL-17F/TNF/IL-1 β . After 48 hour of culture, supernatants were recovered and IL-6 and IL-8 levels were measured by ELISA. A data analysis of microarrays, done on synoviocytes, was realized.

Results IL-17A and IL-17F alone increased significantly both IL-6 and IL-8 production by synoviocytes, with a higher efficiency for IL-17A. For IL-6, TNF and IL-1 β alone had the largest effect on myoblasts, increasing 5 fold the secretion, while for IL-8 it was on skin fibroblasts, increasing at least 5 fold the production. The synergy between IL-17A and TNF was observed on all cells for IL-6 secretion, and for IL-8, only endothelial cells did not show a synergistic effect. For IL-17F and TNF, except endothelial cells, a synergy was also observed, however less powerful than IL-17A/TNF. The effect of the combination IL-17A/IL-1 β or IL-17F/IL-1 β was more dependent on the cell type, with an additive effect for synoviocytes, and a synergistic effect for hepatocytes. The effect of combination of the three cytokines could induce an additive effect as for synoviocytes or a synergistic effect as for skin fibroblasts. The analysis of microarrays showed 1346 regulated probes, with a measured modulation of more than 2 fold, and 279 probes more than 4 fold, with a stronger effect of IL-17A/TNF combination on cell signalization compared to IL-17F/TNF or each cytokine alone.

Conclusions IL-17A and IL-17F act in a similar way on the different cell types, by inducing pro-inflammatory cytokine secretion. The difference was the intensity of the response, where IL-17A induced a higher effect than IL-17F. IL-17A and IL-17F activities were potentiated by the combination with TNF and IL-1 β . The effect of these combinations on cytokine production was dependent on cell types. These differences may explain different response to treatment according to the primary cell type involved in disease.

Disclosure of Interest None declared.

P100

SYSTEMIC AND LOCAL IL-17A AND MIR-146A LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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10.1136/annrheumdis-2018-EWRR2019.88

Career situation of first and presenting author Assistant.

Introduction Interleukin 17 (IL-17) is a proinflammatory cytokine, which has been recognized as an important cytokine in the pathogenesis of rheumatoid arthritis (RA).¹ Altered systemic and local expression of specific microRNAs (miRNAs) was been reported in samples of RA patients.² It has been found that miR-146a was associated with IL-17 expression in IL-17 producing T-cells in the synovium thus indicating its possible role in RA pathogenesis via IL-17 expression.³

Objectives To examine a possible deregulation of the expression levels of miR-146a and levels of IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods Expression levels of miR-146a were determined in matched PB and SF samples of RA patients by relative quantitation method 2^{- $\Delta\Delta C_t$} . As reference control for normalization RNU6B gene was used. Receiver operating characteristic (ROC) curve analysis using RQ values was constructed in

order to evaluate the diagnostic accuracy of miR-146a in PB and SF for distinguishing RA patients from healthy controls (HCs). 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 HCs as well as within the RA group.

Results miR-146a was overexpressed in 70.83% of RA SF ($p=0.007$) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-146a in SF with $AUC=0.769$ (95 CI: $0.600\div0.938$) with 75.0% sensitivity and 72.3% specificity ($p=0.006$). SF levels of miR-146a were overexpressed in 52.17% of the RA 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-146a and IL-17A in RA patients indicates that the local inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-146a and IL-17A suggest the possible relationship between miR-146a expression and the production of IL-17 from IL-17 expressing cells.

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Acknowledgements The study was supported by Grant 60/2013 and Grant 61/2015 from Medical University-Sofia, Bulgaria.

Disclosure of Interest None declared.

P101 SYSTEMIC AND LOCAL IL-17A AND MIR-155 LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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10.1136/annrheumdis-2018-EWRR2019.89

Career situation of first and presenting author Assistant.

Introduction Interleukin 17 (IL-17) is a proinflammatory cytokine, which overproduction promotes the autoimmune reaction in rheumatoid arthritis (RA). Posttranscriptional regulation of IL-17 by specific microRNAs (miRNAs) is of great interest in the recent years. Studies have shown that miR-155 enhances Treg and Th17 cells differentiation and IL-17A production by directly targeting the suppressor of cytokine signaling (SOCS) 1.^{1,2}

Objectives To examine a possible deregulation of the expression levels of miR-155 and levels of IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods Expression levels of miR-155 were determined in matched PB and SF samples of RA patients by relative quantitation method $2^{-\Delta\Delta C_t}$. As reference control for normalization RNU6B gene was used. Receiver operating characteristic (ROC) curve analysis using RQ values was constructed in

order to evaluate the diagnostic accuracy of miR-155 in PB and SF for distinguishing RA patients from healthy controls (HCs). 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 HCs as well as within the RA group.

Results miR-155 was overexpressed in 79.17% of RA SF ($p=1.63 \times 10^{-4}$) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-155 in SF with $AUC=0.858$ (95 CI: $0.757\div0.959$) with 81.3% sensitivity and 81.8% specificity ($p=2.3 \times 10^{-4}$). SF levels of miR-155 were overexpressed in 76.09% of the RA 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-155 and IL-17A in RA patients shows that the inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-155 and IL-17A confirm the data about the possible role of miR-155 in regulating the production of IL-17.

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Acknowledgements The study was supported by Grant 60/2013 and Grant 61/2015 from Medical University-Sofia, Bulgaria.

Disclosure of Interest None declared.

P102 HIGH DIMENSIONAL PROFILING OF REGULATORY T CELLS IN SPONDYLOARTHRITIS REVEALS CELLULAR HETEROGENEITY

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10.1136/annrheumdis-2018-EWRR2019.90

Career situation of first and presenting author Student for a master or a PhD

Introduction The Spondyloarthritides (SpA) are immune-mediated conditions characterised by spinal and joint inflammation. Numerous studies implicate Th17 lymphocytes in pathogenicity but few reports exist on the role played by regulatory T cells (Tregs) in the disease.

Objectives

- To describe Treg frequency and phenotype in SpA patients compared to controls.
- To identify differential expression of trafficking molecules and activation markers within Tregs at the inflammatory site.

Methods A total of 61 patients with SpA (38 with AS, 23 with PsA) and 16 age-matched healthy controls were recruited. Peripheral blood (PB) and paired synovial fluid (SF) mononuclear cells (n=8) were stained for with 3 multicolor flow cytometry panels, including a total of 35 surface and