BGP-15 treated group when compared to the control group. No significant differences were revealed between anti-collagen antibody levels or in the distribution of T-cells, B-cells, dendritic cells and monocyte/macrophages harvested from draining lymph nodes, suggesting an effect predominantly involving the innate immune system.

Conclusions Our results demonstrate that the novel chaperone-inducer BGP-15 has a profound prophylactic and therapeutic effect on autoimmune arthritis, likely due to an effect on the effector phase.

A9.10 NEUTRALISATION OF ACPA – A WAY TO GO?

doi:10.1136/annrheumdis-2013-203223.10

[1]C Cerqueira, [1]E Ossipova, [1]M Hansson, [2]L Mathsson, [1]L Klareskog, [2]J Rönnelid, [1]P-J Jakobsson. *[1]Department of Medicine, Unit of Rheumatology, Karolinska University Hospital, Stockholm, Sweden; [2]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*

Background and Objectives In a previous study, we have identified endogeneously citrullinated sites in fibrinogen from RA synovial tissue (Hermansson, *et al*, 2010 in *Proteomics-Clin Appl*). Within the alpha chain, Arg573 and Arg591 were found citrullinated with an occupancy rate in the range of 1–2% and in the β-chain, Arg72 and Arg74 were also found citrullinated. We now demonstrate that these citrullinated residues are autoantigenic as well as demonstrate that peptides containing these epitopes can be used as probes for development of ACPA neutralising compounds.

Materials and Methods The autoantigenic potential was investigated using the Phadia's ImmunoCAP ISAC® system. Citrullinated and unmodified fibrinogen peptides were immobilised onto a glass slide in an arrayed fashion and serum from 404 CCP positive and 532 CCP negative RA patients and 461 healthy controls from the EIRA cohort were tested. We also assayed the identified citrulline fibrinogen peptides for their ability to prevent purified ACPA (Ossipova, *et al*, 2012 submitted) to bind to CCP (CCPlus® ELISA, Euro-Diagnostica AB). Peptides were individually or in combinations incubated with different ACPA pools and the blocking efficiency was expressed as percent of inhibition and IC50. Corresponding arginine peptides were used as controls.

Results We found that 31% (87% are CCP positive) of patients were positive to Cit573 peptide. For the Cit591 peptide, the corresponding numbers were 10% (65%), for the Cit74 peptide 28% (68%) and for the Cit72 peptide 20% (68%). Interestingly, citrullinated 573 and Cit591 peptides revealed a maximum of 77% and 48% ACPA inhibition, respectively. When equally mixed, these peptides displayed an additive higher degree of ACPA neutralisation (84%). In contrast, Cit74 and Cit72 peptides reached a more modest maximum inhibition of 26% and 30%, respectively. This experiment was repeated using a different set of ACPA pool and then the efficiencies were lower for Cit573 (47%) but similar for Cit591 (51%). Logically, the efficiency of specific citrullinated compounds will depend on the individual ACPA specificities.

Conclusions Here we demonstrate extensive autoantibody reactivity against in vivo citrullinated fibrinogen epitopes found in RA synovial membranes. These peptides can now be used as additional biomarkers for studies of ACPA sub-specificity profiles as recently reported (Brink, *et al*, 2012 *A&R*, in press). We also demonstrate that these citrullinated peptides can be used as neutralising agents blocking a significant portion of ACPA binding to CCP. These results open novel possibilities for the design of personalised ACPA blockers preventing for instance the osteoclastogenesis and bone loss induced by ACPA (Harre, *et al*, 2012 *JCI*).

A9.11 NF-kB INDUCING KINASE (NIK) IS A KEY REGULATOR OF INFLAMMATION-INDUCED ANGIOGENESIS

doi:10.1136/annrheumdis-2013-203223.11

[1]AR Noort, [1]KPM van Zoest, [2]P Koolwijk, [1]PP Tak, [1]SW Tas. *[1]Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, The Netherlands; [2]Department of Physiology, Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, Amsterdam, The Netherlands*

Background In rheumatoid arthritis (RA) synovial tissue (ST) angiogenesis can be observed already in the earliest phase of disease. The chemokine CXCL12, which is induced via the non-canonical nuclear factor-kappaB (NF-kB) pathway, plays an important role in angiogenesis, lymphocyte transendothelial migration, and the homing of endothelial progenitor cells. Therefore, the non-canonical pathway, with its key mediator NF-kB inducing kinase (NIK), may play an important role in pathological angiogenesis and the perpetuation of synovial inflammation in RA.

Objective To study the role of non-canonical NF-kB signalling in pathological angiogenesis in RA.

Methods Expression of NIK and CXCL12 in RA ST was evaluated using immunofluorescence microscopy (IF). Angiogenesis was studied in endothelial cells (EC) in vitro using the tube formation assay and ex vivo by comparing WT and $NIK^{-/-}$ mice in the aortic ring assay. Physiological (developmental) angiogenesis was evaluated by isolectin B4 staining of the retina followed by confocal microscopy. The contribution of NIK to synovial angiogenesis was studied in vivo in antigen-induced arthritis (AIA).

Results NIK, p52 and CXCL12 were highly expressed in EC in RA ST, mainly in small (newly formed) blood vessels. Stimuli that induce non-canonical NF-kB signalling (lymphotoxin (LT), LIGHT, and CD40L) significantly enhanced in vitro tube formation 2.5-fold ($p < 0.05$), which could be completely blocked by siRNA targeting NIK or IKKα. Aortic rings from WT and $NIK^{-/-}$ mice showed normal TNF- and VEGF-induced microvessel outgrowth. In contrast, whereas non-canonical NF-kB stimuli induced microvessel outgrowth in WT mice (unstim 29.94 ± 6.08 versus LT 159.1 ± 50.24 versus LIGHT 110.3 ± 17.68 (mm^2) $p < 0.05$), no microvessel outgrowth was observed in aortic rings from $NIK^{-/-}$ mice (unstim 28.74 ± 15.89 versus LT 45.9 ± 16.71 versus LIGHT 43.41 ± 15.73 (mm^2)). In line with this, $NIK^{-/-}$ mice exhibited normal developmental angiogenesis in the retina, but a 50% reduction in pathological angiogenesis in synovial inflammation (blood vessels in synovial tissue WT 20 ± 5.07 versus $NIK^{-/-}$ 10.2 ± 3.02).

Conclusions NIK is preferentially expressed in EC in RA ST and non-canonical NF-kB signalling in EC results in enhanced angiogenesis in vitro. $NIK^{-/-}$ mice exhibited normal developmental and VEGF-induced angiogenesis, but reduced pathological angiogenesis in AIA. These findings point towards an important role of the non-canonical NF-kB pathway in pathological angiogenesis associated with chronic (synovial) inflammation. This could be exploited for the development of future new therapies for *RA*.

A9.12 NON-CANONICAL NF-KAPPAB SIGNALING INDUCES A PROANGIOGENIC RESPONSE IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS AND ENDOTHELIAL CELLS

doi:10.1136/annrheumdis-2013-203223.12

[1,2]CX Maracle, [1,2]AR Noort, [1,2]KPM van Zoest, [1,2]SW Tas. *[1]Divison of Clinical Immunology and Rheumatology; [2]Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands*

Background Synovial fibroblasts (SF) play a pivotal role in the pathophysiology of rheumatoid arthritis (RA). RASF not only promote inflammation and cartilage destruction of the joint, but also stimulate angiogenesis. Angiogenesis allows for increased leukocyte

entry into the inflamed synovium and is regarded as a switch from acute to chronic inflammation. The non-canonical nuclear factor kappaB (NF-kB) pathway, with its main regulator NF-kB inducing kinase (NIK), may play a central role in this process.

Objectives To determine the effect of non-canonical NF-kB signalling on pro-angiogenic gene expression in RASF and on the angiogenic potential of human umbilical vein endothelial cells (HUVEC).

Methods RASF were stimulated with lymphotoxin $\alpha_1\beta_2$ (LT) or LIGHT to activate non-canonical NF-kB signalling, and/or TNF to selectively activate the canonical NF-kB pathway. To effectively block the non-canonical pathway, a dominant negative NIK expressing adenovirus (Ad.NIKdn) was used. Changes in pro-angiogenic gene expression were measured by RT-PCR. Furthermore, to determine the effect on EC proliferation, RASF and HUVEC were co-cultured in the absence or presence of LT, LIGHT, TNF or VEGF EC were visualised through immunohistochemical staining of CD31, which was then semi-quantitatively scored.

Results Gene expression analysis of RASF revealed an increase in mRNA levels of VCAM-1 and IL-6 after stimulation with LT, LIGHT and TNF. Increased expression of IL-8 and MMP-3 was also observed in cells treated with both TNF and LT or LIGHT. These levels were attenuated in cells transduced with Ad.NIKdn prior to stimulation, indicating that the increased expression levels were at least in part non-canonical NF-kB dependent. CCL2 and bFGF were expressed continuously by RASF regardless of stimulation. In the co-culture, proliferation levels of EC increased under all stimulation conditions, with LIGHT inducing almost a 2-fold increase ($p < 0.05$), which was comparable to VEGF ($p < 0.05$).

Conclusions RASF contribute to synovial angiogenesis through the expression of adhesion molecules, cytokines, chemokines, matrix remodelling enzymes and growth factors. We demonstrate that the non-canonical NF-kB pathway plays an important role in this process by regulating pro-angiogenic genes and promoting EC proliferation. Further investigation of this pathway could lead to novel non-canonical NF-kB blocking therapeutics that inhibit angiogenesis in RA, thereby halting disease progression.

A9.13 TNF-INDUCED- PROTEIN TYROSINE PHOSPHATASE NONRECEPTOR TYPE 2 (PTPN2) AS A NEGATIVE REGULATOR OF INFLAMMATION IN RHEUMATOID ARTHRITIS

doi:10.1136/annrheumdis-2013-203223.13

[1]Borbala Aradi, [1]Maria Filkova, [2]Stephanie Kasper, [1]Kerstin Klein, [2]Michael Scharl, [1]Beat A Michel, [1]Renate E Gay, [3]Edit I Buzas, [1]Steffen Gay, [1]Astrid Jüngel. *[1]Center of Experimental Rheumatology, University Hospital Zürich and Zürich Center for Integrative Human Physiology, Zürich, Switzerland; [2]Division of Gastroenterology and Hepatology, University Hospital Zürich and Zürich Center for Integrative Human Physiology, Zürich, Switzerland; [3]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary*

Background and Objectives Protein Tyrosine Phosphatase Non-receptor Type 2 (PTPN2) is a protein tyrosine phosphatase that plays a role in the development of autoimmune diseases. PTPN2 function has not been studied in rheumatoid arthritis (RA), although single nucleotide polymorphisms within the gene have been described to be associated with RA in genome wide association studies. Considering the involvement of PTPN2 in the regulation of key inflammatory pathways, our aim was to analyse the expression and function of PTPN2 in RA synovial fibroblasts (RASF).

Materials and Methods The expression of PTPN2 was assessed in synovial tissue and fibroblasts (passage 4–10) from patients with RA and osteoarthritis (OA) using immunohistochemistry, real-time PCR (w/o tumour necrosis factor α (TNFα), IL1β, LPS and hypoxia) and Western blotting. PTPN2 was silenced with silencing RNA. Levels of IL-6 and IL-8 expression were measured by commercially available ELISA in cell culture supernatants after silencing PTPN2 in RASF w/o stimulation with tumour necrosis factor α (TNFα). Apoptosis of RASF was evaluated by AnnexinV staining using flow cytometry after stimulation with TNF-related apoptosis-inducing ligand (TRAIL, 20 ng/ml) for 24 hours.

Results In RA synovial tissue, compared with OA, we observed a stronger staining of PTPN2 in both the lining and the sublining layer by immunohistochemistry. On mRNA level we confirmed this overexpression in RA synovial tissue (2.0 fold, n = 4–5). In isolated RASF the constitutive mRNA level of PTPN2 was higher than in OASF (1.6 fold, $p < 0.01$, n = 10–16).

Levels of PTPN2 were further upregulated in RASF after stimulation with inflammatory cytokines such as TNFα (10 ng/ml, 24 hours, 3.1 fold, $p < 0.05$, n = 4), TNFα and IL-1β (1 ng/ml, 2.3 fold, n = 5), LPS (100 μg/ml, 24 hours, 1.9 fold, n = 5) and by 1% hypoxia (1.3 fold, n = 3). Accordingly, basal PTPN2 protein expression was 2.0 fold higher in RASF than in OASF (n = 4) and TNFα upregulated levels of PTPN2 (1.7 fold). PTPN2-deficient RASF produced 2.4 times more IL-6 than scrambled siRNA transfected cells (mean ± SD pg/ml 11412 ± 6313 versus 28133 ± 12734, n = 3). On the other hand, levels of IL-8 were not affected (35800 pg/ml versus 24330 pg/ml, n = 3). Furthermore, after silencing, 34% increase in TRAIL-induced apoptosis was detected in RASF (n = 5) compared to scrambled controls.

Conclusions Our findings indicate that PTPN2, known to be involved in the pathogenesis of several autoimmune diseases, could be an important negative regulator of inflammation in RASF.

Acknowledgement This work was supported by IMI BTCure, IAR and Masterswitch-FP7.

A9.14 REGULATORY T CELL ABNORMALITIES IN PATIENTS WITH SLE SUGGEST AN IL-2-BASED IMMUNOTHERAPY

doi:10.1136/annrheumdis-2013-203223.14

[1,2]C von Spee, [2]N Wassermann, [1,2,*]JY Humrich, [1,2,*]G Riemekasten. *[1]German Rheumatism Research Centre, Berlin, Germany; [2]Charité University Hospital, Rheumatology and Clinical Immunology, Berlin, Germany.*

**These authors contributed equally*

Background Phenotypic and quantitative abnormalities of regulatory T cells have been described in association with systemic lupus erythematosus (SLE). Further, an impaired production of IL-2, the essential cytokine for Treg homeostasis, has been described for T cells from SLE patients. Here, we aim to substantiate the link between IL-2 deficiency and Treg abnormalities in SLE and to provide the basis for an IL-2 based immunotherapy.

Methods Phenotype, frequency and homeostatic status of Foxp3+CD127lo Treg and conventional Foxp3- T cell (Tcon) subsets were analysed by multi-colour flow-cytometry of PBMCs from SLE patients and healthy donors ex vivo and after in vitro low-dose IL-2 treatment. Disease activity was determined according to the SLE activity index (SLEDAI). Two-tailed Mann-Whitney U test or 2-way ANOVA test were used for statistical analysis between patient or treatment groups, Spearman's rank coefficient was used to calculate correlations with disease activity.

Results The frequency of CD25+ cells among Treg was significantly reduced in SLE patients compared to HC. Analysis of Ki67 expression revealed that proliferation was significantly increased in Tcon from SLE patients, resulting in a reduced Treg to Tcon proliferation ratio in SLE patients. The proliferation ratio correlated positively with the frequency of CD25+ Treg and inversely with disease activity. Treatment of SLE PBMCs with low-dose IL-2 in vitro resulted in increased frequencies of CD25+ cells among Treg and increased CD25 expression levels on Treg. Treg, but not Tcon proliferation was significantly increased under low-dose IL-2 treatment compared to untreated controls.