Background Local bone destruction in rheumatoid arthritis, psoriasis arthritis or ankylosing spondylitis is a serious health burden and the major cause of disability and severely reduced quality of life in these diseases. This damage to the bony structures is exclusively mediated by a special cell type, the osteoclast (OC). Therefore, it is important to understand factors and pathways regulating the generation of OCs under inflammatory conditions. As PTEN is a lipid phosphatase and one of the main antagonists of the PI3-kinase, we analysed the impact of the PI3-Kinase/PTEN axis on OC generation and bone biology in an animal model of inflammatory bone loss.

Methods We induced osteoclastogenesis in wt and PTEN deficient bone marrow cells and measured the generation of OCs, their resorptive capacity and induction of OC differentiation markers in vitro. Moreover, we analysed mice with a monocyte/macrophage-specific deletion of PTEN (myeloid specific PTEN$^{-/-}$) by bone histomorphometry and crossed these mice into hTNFtg animals.

Results We show that myeloid specific PTEN$^{-/-}$ mice have increased osteoclastogenesis in vitro and in vivo when compared to wild-type animals. However, under non-inflammatory conditions, enhanced osteoclastogenesis did not result in systemic bone loss in vivo. However, when we crossed myeloid specific PTEN$^{-/-}$ into hTNFtg mice we found significantly decreased grip strength scores in myeloid specific PTEN$^{-/-}$/hTNFtg mice compared to wt hTNFtg mice. Joint swelling scores, however, were not different between both groups. In line, myeloid specific PTEN$^{-/-}$/hTNFtg mice displayed enhanced local bone destruction as well as OC formation in the inflamed joints, whereas the extent of synovial inflammation was not different between the groups. Analysis of the synovial membranes of wt and myeloid specific PTEN$^{-/-}$ animals revealed similar relative compositions of the cellular infiltrate including macrophages, which serve as OC precursors. This suggests that increased capacity for osteoclastogenic differentiation rather than enhanced recruitment of precursor cells is responsible for the enhanced local generation of OCs.

Conclusions Taken together, these data demonstrate that sustained PI3-Kinase activity in myeloid cells specifically elevated the osteoclastogenic potential of these cells, leading to enhanced inflammatory local bone destruction. Therefore, targeting the PI3-Kinase pathway therapeutically may be especially useful for the prevention of structural joint damage.

A9.8 LOW-DOSE IL-2 THERAPY SELECTIVELY EXPANDS REGULATORY T CELLS AND AMELIORATES ESTABLISHED DISEASE IN (NZBxNZW) F1 LUPUS MICE

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Background Our previous studies in the (NZBxNZW) F1 model provide strong rationales for an IL-2 based immunotherapy of lupus in order to restore regulatory T cell (Treg) mediated tolerance that is impaired due to an acquired IL-2 deficiency (Humrich *et al*, 2010). However, because of its pleiotropy, other cells than Treg can be activated by IL-2 in a dose dependent manner, which may induce unwanted side effects or even trigger autoimmunity.

Objectives To determine an optimal regimen for an IL-2 based immunotherapy that is capable to induce a sufficient expansion of CD4+Foxp3+Treg in vivo while only marginally affecting other cells, and that most efficiently influences active disease in the (NZBxNZW) F1 model for lupus.

Methods Recombinant mouse IL-2 at various single doses was injected subcutaneously either into young or diseased (NZBxNZW) F1 mice every day for the duration of five days as induction therapy. After the induction phase, IL-2 injections were continued every 4 days until the end of the experiment. Control animals received an equal amount of PBS (carrier). Cells from lymphoid organs and peripheral blood were analysed by flow cytometry at different time points throughout the study. In addition survival and clinical parameters (weight, proteinuria, leukozyturia, autoantibodies) were analysed during IL-2 therapy of diseased mice for regimens with the single dosages of 5 ng/g and 25 ng/g body weight.

Results We found that the low-dose IL-2 regimen with a single dose of 5 ng/g body weight sufficiently promoted the expansion of CD4+Foxp3+Treg, while not or only marginally affecting CD4+ conventional T cells (Tcon) and other potentially harmful cells. Although higher doses of IL-2 resulted in a more pronounced proliferation and expansion of Treg, this was accompanied by a considerable increase in CD4+ memory/effector Tcon and NK/NKT cells. Clinically, regimens with both 5 ng/g and 25 ng/g were almost equally sufficient to influence nephritis and to decrease mortality in mice with established disease.

Conclusions These studies show that a low-dose IL-2 regimen selectively targets Treg and is clinically effective and also safe in murine lupus providing essential rationales for the clinical introduction of an IL-2 based immunotherapy in SLE.

References

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A9.9 TREATMENT WITH BGP-15, A NOVEL INSULIN SENSITISER ATTENUATES COLLAGEN-INDUCED ARTHRITIS IN DBA/1 MICE

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Background and Objectives BGP-15, a small synthetic hydroxylamine derivative is a member of a new class of insulin-sensitising medications also known as chaperone-inducers. Beside its beneficial effects on glycemic control and insulin sensitivity in patients with Type 2 diabetes, BGP-15 is known to induce heat shock protein Hsp72 and heat shock transcription factor HSF1, which in turn are involved in joint inflammation. Moreover, BGP-15 also inhibits poly-ADP-ribose polymerase (PARP) and the phosphorylation of c-JUN N-terminal kinase via Hsp72 overexpression. Therefore it might also play a role in the regulation of inflammatory joint disease. Our objective was to evaluate the in vivo effects of BGP-15 on collagen-induced arthritis (CIA) in DBA/1 mice.

Materials and Methods Arthritis was induced by intradermal injection of bovine type II collagen (bCII) and incomplete Freund's adjuvant (CFA) in male DBA/1 mice. BGP-15 was administered either one week prior to the first immunisation (prophylactic experiment, n:14 in both groups) or upon the appearance of symptoms (therapeutic experiment, n:12 in both groups) in drinking water. Arthritis incidence and severity was assessed for 28 days following the second immunisation (boost) with bCII and CFA on day 21. Histological evaluation was carried out on hind paws using Osteomeasure® software. Anticollagen antibodies were measured by enzyme-linked immunosorbent assay. The cellular composition of the draining lymph nodes was measured by flow cytometry.

Results BGP-15 significantly reduced the incidence of CIA by 28% and also reduced both paw swelling ($p \leq 0.01$) and grip strength ($p \leq 0.05$) in the prophylactic experiment. In the therapeutic experiment BGP-15 significantly attenuated both paw swelling ($p \leq 0.01$) and grip strength ($p \leq 0.05$). Histological evaluation of the hind paws demonstrated reduced area of inflammation ($p \leq 0.05$), area of erosion ($p \leq 0.01$) and number of osteoclasts ($p \leq 0.05$) in the

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?

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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

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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

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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