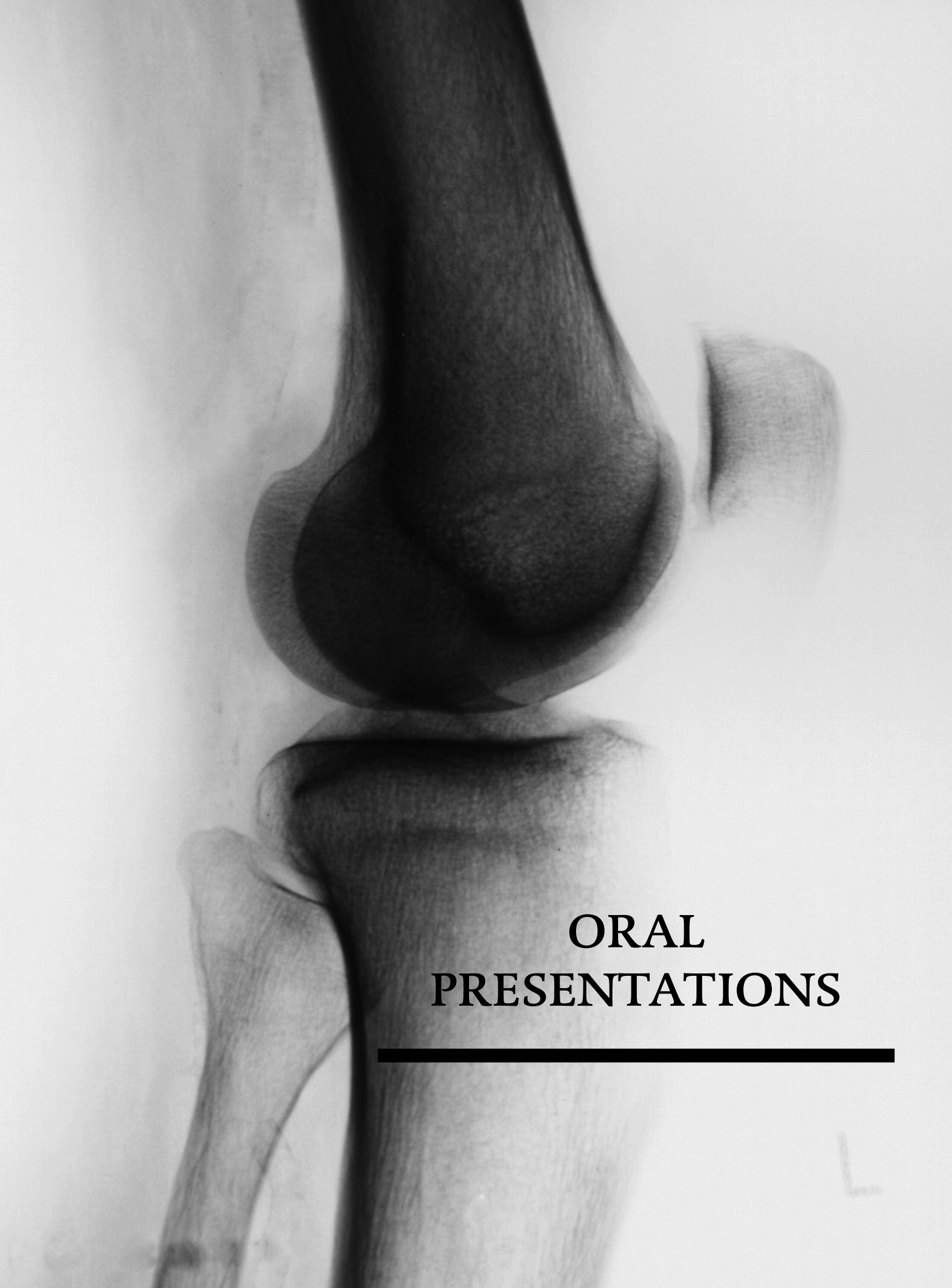

ORAL PRESENTATIONS

Oral Presentations

ACTA REUMATOL PORT. 2014:39:17-25 (SUP)

01

STROMAL OVEREXPRESSION OF TRANSMEMBRANE TNF INDUCES EXPERIMENTAL SPONDYLOARTHRITIS IN MICE

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Background: The failure of TNF blockers to prevent osteoproliferation in SpA patients raised the concept that inflammation and osteoproliferation are uncoupled in SpA. However, inflammation and osteoproliferation are linked in HLA-B27 rats, high CRP is associated with radiographic progression in axial SpA, and NSAID treatment can retard osteoproliferation.

We propose that inflammatory mediators, distinct from soluble TNF, drive pathologic osteoproliferation in SpA. Based on our observations on soluble versus transmembrane TNF (tmTNF) expression in SpA synovitis, we explored if and how tmTNF drives experimental SpA.
Methods: tmTNF mice[1] were studied over time for SpA development. Joints were collected and analyzed for inflammation and osteoproliferation.

To assess the contribution of stromal versus hematopoietic tmTNF overexpression, tmTNF mice and WT mice were lethally irradiated and received bone marrow cells (BM) from either WT or tmTNF mice. Mice were evaluated for 16 weeks until sacrificed for histologic and radiographic analysis.
Results: tmTNF mice (100%; n>50) spontaneously developed arthritis and spondylitis, starting at 4 weeks of age and progressing over time. Arthritis was characterized by inflammation of synovium and enthesis. Hypertrophic chondrocytes were observed outside the bone in the connective tissue next to the inflammation. In spondylitis, inflammation was found in connective tissue located at the junction of the annulus fibrosus with the vertebral bone. Hypertrophic chondrocytes were observed at the edge of the vertebral body, in conjunction with ongoing inflammation.

In the functional experiments, irradiated tmTNF mice receiving tmTNF BM developed arthritis and spondylitis with 100% incidence 3 weeks after transplantation. tmTNF mice receiving WT BM also developed disease with the same incidence, onset and severity as the control group. In sharp contrast, WT mice that received tmTNF BM did not develop any arthritis, and spondylitis occurred less frequently (66%) and later (10 weeks after BMT) compared to the control group.
Conclusions: tmTNF overexpression induces experimental SpA with osteoproliferation, indicating that inflammatory mediators can indeed drive osteoproliferation. These data indicate the relevance of transmembrane TNF and the role of the stromal compartment in the pathophysiology of SpA.

REFERENCE:

1. Alexopoulou L, et al. Eur J Immunol 1997; 27(10):2588-92.

02

INDUCTION OF IL-10 EXPRESSION IN HUMAN CD4+ T CELL SUBSETS BY BIOLOGICAL THERAPEUTICS

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Background: Recent research demonstrates that pro-inflammatory cytokine-producing CD4+ T cells can acquire anti-inflammatory potential characterised by expression of interleukin-10 (IL-10). We recently showed that TNF blockade induces IL-10 expression in human CD4+ T cells *in vitro* and *in vivo* (Evans *et al. Nature Communications* 2014). Here we examine whether blocking other pro-inflammatory cytokines (IL-1, IL-6) or CD80/CD86 co-stimulation also induces IL-10 and investigate the effects of anti-TNF on proliferative responses of CD4+ T cells *in vitro*.
Material and Methods: CD4+ or CD4+CD45RO+ T

cells and CD14+ monocytes were isolated from peripheral blood from healthy volunteers and co-cultured for 3 days with anti-CD3 mAb, in the absence/presence of adalimumab (anti-TNF), tocilizumab (anti-IL-6R), abatacept (CTLA-4-Ig), anti-IL-1R1 or recombinant TNF-α. Following re-stimulation (PMA and ionomycin in the presence of GolgiStop), intracellular cytokine staining was performed.

Proliferative responses were assessed by dye dilution assays. Gene expression profiling of anti-TNF-exposed IL-17+ (Th17) and IFN- + (Th1) CD4+ T cells was followed by gene ontology analysis of commonly differentially expressed genes.

Results: Anti-TNF addition induced IL-10 expression in pro-inflammatory cytokine-producing CD4+ T cells and conversely, TNF-α addition suppressed IL-10 expression in these cells. IL-1R1 blockade also increased IL--10 expression in CD4+ T cell subsets, whilst neither tocilizumab nor abatacept had this effect. In the presence of anti-TNF CD4+ T cell proliferative responses were reduced. Pathway analysis of genes commonly regulated by anti-TNF in Th1 and Th17 cells indicated enrichment for cell cycle-associated functional annotations.

Discussion: Our data demonstrate that blockade of either TNF-α or IL-1β, but not IL-6 signalling, induces IL--10 expression in pro-inflammatory cytokine-producing CD4+ T cell subsets. TNF blockade also impairs *in vitro* proliferative responses of anti-CD3-stimulated CD4+ T cells. Current experiments are aimed at exploring whether there is a link between IL-10 induction and reduced proliferation in anti-TNF-exposed CD4+ T -cells.

03

NOVEL ROLE FOR MIR-125B IN REGULATING METABOLIC ADAPTATION OF MONOCYTES TO INFLAMMATION

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Aim: Abnormal mitochondria metabolism and innate immune responses participate in the pathogenesis of many inflammatory disorders. However, regulation of mitochondrial activity to control survival and cell death in monocytes/macrophages is only poorly understood.

Materials and Methods: We used gain- and loss-of--function experiments to evaluate the effect of miR--125b on the human monocytic cell line THP-1, as well as primary sorted CD14+ monocytes, under steady state, inflammatory or M1/M2 polarizing conditions. A transcriptome analysis was performed to identify putative targets that were validated using a luciferase reporter construct containing the 3'UTR of candidate genes, followed by RT-qPCR and western blots. Apoptosis was analyzed by Caspase 3/7 assays. Monocyte oxygen consumption rate was investigated using the Seahorse Extracellular Flux Analyzer. TOM20 and MitoTracker stainings were used to monitor the mitochondrial network, quantified by Cellomics ArrayScan VTi platform.

Results: We showed that miR-125b attenuates the activity of the mitochondrial respiratory chain through BIK silencing, and promotes the elongation of mitochondrial network through MTP18 targeting, without impacting autophagy, in the human monocytes. Proinflammatory activation is associated with a concomitant increase in miR-125b expression, decrease in BIK and MTP-18 expression, reduced oxidative phosphorylation and enhanced mitochondrial fusion. LPS upregulates miR-125b expression and LPS-induced repression of BIK expression and of mitochondrial respiration are impeded by introduction of synthetic miR--125b antagonist. Furthermore, expression of M1-associated transcripts as well as mitochondrial dynamics and energy metabolism are induced upon ectopic expression of miR-125b in THP-1 cells.

Conclusion: Altogether, our data reveal a novel role for miR-125b in controling mitochondrial metabolism and dynamics by targeting BIK and MTP18 respectively, two novel cellular target proteins involved in maintaining the mitochondrial integrity. These findings not only suggest a novel function for miR-125b in regulating metabolic adaptation of cells to inflammation but also unravel new molecular mechanisms for its pro-apoptotic role.

04

A ROLE FOR ENDOGENOUS IL-22 BINDING PROTEIN IN BLOCKING BENEFICIAL ACTIONS OF IL-22 IN EXPERIMENTAL ACUTE COLITIS AND CROHN'S DISEASE

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Crohn's disease (CD) and ulcerative colitis (UC), the two forms of inflammatory bowel diseases (IBDs), are disorders of the gastrointestinal tract that result from abnormal immune responses against the gut microflora. Although several studies have reported elevated levels of IL-22 in both active CD and UC, their pathophysiological significance remains unclear. In addition, IL-22 actions are regulated by a potent soluble inhibitor named IL-22BP whose expression and sources have not been assessed in IBDs. Here, we showed in human that, as in rodents, strong mRNA expression is observed in gut *lamina propria* conventional DCs.

However, eosinophils actually represent the most abundant producer cells of IL-22BP in human healthy gut. IL-22BP production by eosinophils was much greater in active colonic CD than in UC. This suggested a differential regulation of IL-22 actions in these diseases that was further sustained by a lower induction of IL-22-inducible antimicrobial peptides (AMPs) in CD as compared to UC. Using IL-22BP-deficient rats we further showed that endogenous IL-22BP hinders IL-22 protective actions on the gut epithelium during acute colitis. To conclude, this work provides new insights on the role of the IL-22/IL-22R pathway in CD and UC, suggesting possible different actions owing to IL-22BP regulation. It also suggests that IL-22 actions could be therapeutically modulated through direct targeting of IL-22BP.

05

T FOLLICULAR HELPER (TFH) AND FOXP3+ T FOLLICULAR REGULATORY (TFR) CELLS HAVE A DIFFERENT TCR REPERTOIRE

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Introduction: Germinal centers (GC) are key structures where B cells are selected to produce high affinity immunoglubulins of the adequate class. This selection is dependent of B cell interactions with specialized T follicular helper (Tfh) cells that provide help for class switch recombination, somatic hypermutation, and selection signals to germinal center B cells. It was recently described that the GC reaction (GCR) is controlled by specialized Foxp3+ T follicular regulatory (Tfr) cells. These cells regulate the size and magnitude of the GCR, as well as the production of autoantibodies following chromatin immunization.
Materials and Methods: We analysed the TCR diversity of different T cell populations, as well as adoptive transfers of antigen-specific TCR-transgenic T cells to address the hypothesis that the TCR repertoire of Tfh and Tfr cells arising following immunization is different.
Results: We found that Tfh cells isolated from draining lymph nodes of mice immunized with non-self antigens are enriched in TCRs specific for the immunizing antigen. However, Tfr cells from the same nodes do not share the same TCR composition, as they retain greater TCR diversity (regarding Vβ usage) and lower representation of TCRs specific for the immunizing non-self antigen, resembling the TCR repertoire of natural Foxp3+ Treg cells.
Discussion: The observed difference in TCR usage between Tfh and Tfr cells is a likely consequence of their different immediate precursors: while Tfh cells differentiate from uncommitted naive T cells, Tfr cells originate from thymic-derived natural Treg cells known to have a repertoire bias for self antigens.
Conclusion: Our results suggest that participation of

Tfr cells in limiting autoantibody-associated autoimmunity may be dependent of a TCR repertoire skewed towards self antigens.

06

RECEPTOR ACTIVATING NF-ΚB LIGAND (RANKL) IS A CONSTITUTIVE INTRACELLULAR PROTEIN IN RESTING HUMAN BASOPHILS AND IS STRONGLY INDUCED ON THEIR SURFACE BY INTERLEUKIN 3

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Receptor Activating NF-kB ligand (RANKL) is a member of the TNF superfamily that plays a pivotal role in bone homeostasis as being the major osteoclastogenesis factor. RANKL also has pleiotropic effects in the immune system in which it is expressed by activated T and B cells and some innate lymphoid cells. RANKL--RANK interactions mediate lymph node organogenesis and immunoregulatory functions in autoimmune disease and carcinogenesis as well as cross talk between the immune system and bone. In this study, we show that basophils were the strongest RANKL mRNA-expressing cells amongst major leukocyte subsets in human blood. RANKL was preformed as an intracellular protein in resting basophils and was rapidly and strongly expressed on their surface upon stimulation with IL-3, but not other stimuli. This expression was stable for at least 6 days. Activated basophils could also release soluble RANKL in small quantities upon interaction with DCs or monocytes. In the blood, basophils were the sole cells to express membrane RANKL in response to IL-3. This study indicates that basophils should be considered as new players in the pleiotropic and complex RANKL-RANK interaction system and suggests a role for RANKL in the interaction between basophils and immune cells in inflammatory allergic tissues and secondary lymphoid organs.

07

TARGETING SYNOVIAL MAST CELLS IN SPONDYLOARTHRITIS: A PROOF-OF-CONCEPT STUDY WITH THE TYROSINE KINASE INHIBITOR NILOTINIB

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Introduction: Immunopathological studies on synovitis recently identified mast cells as potential therapeutic target in spondyloarthritis (SpA). [*Noordenbos et al. Arthritis Rheum 2012*] Mast cells can be targeted by inhibiting c-Kit, which is a target of the tyrosine kinase inhibitor nilotinib. This study aimed to evaluate the immunomodulating and clinical effects of nilotinib in SpA.
Materials and Methods: 28 patients with active peripheral and/or axial SpA were included in a randomized, double-blind, placebo-controlled clinical trial and were treated 1:1 with nilotinib or placebo for 12 weeks, followed by an open label extension for 12 weeks. Paired synovial biopsies, serum sampling and assessment of clinical symptoms were performed serially.
Results: In peripheral SpA (n=13) synovial inflammation was reduced after 12 weeks of nilotinib treatment as evidenced by histopathology (decrease in CD68+ and CD163+ macrophages and mast cells). Also, compared to placebo mRNA expression of c-Kit (p=0.037) and pro-inflammatory cytokines such as IL-6 (p=0.024) were reduced, paralleled by a decrease in serum inflammation biomarkers such as CRP (p=0.024) and calprotectin (p=0.055). Clinical parameters such as patient's global assessment of disease activity (p=0.031) and Ankylosing Spondylitis Disease Activity Score (p=0.031) improved upon 12 weeks of nilotinib but not placebo treatment, which was further augmented at week 24. In contrast, neither serum inflammation biomarkers nor clinical parameters improved upon nilotinib treatment in axial SpA. One serious adverse event occurred, which was considered unrelated to nilotinib. There were no unexpected safety signals in comparison with large scale data on nilotinib in chronic myeloid leukemia.
Conclusions: This study supports the concept that

mast cells can contribute to synovial inflammation in SpA and that tyrosine kinase inhibition targeting these cells has a biological and clinical immunomodulatory effect in peripheral but not axial SpA. This supports further evaluation of nilotinib and other drugs targeting mast cells in larger trials in peripheral SpA.

08

RHEUMATOID ARTHRITIS PATIENTS HAVE ALTERATIONS IN INHERENTLY AUTOREACTIVE 9G4+ B-CELL SUBPOPULATIONS IN PERIPHERAL BLOOD

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Introduction: The rat monoclonal antibody 9G4 recognizes B-cells with VH4-34-encoded B-cell receptors (9G4+B-cells) and also secreted immunoglobulins. In healthy individuals, 9G4+B-cells present in splenic marginal zones and tonsils are excluded from germinal centre reactions. In rheumatoid arthritis (RA) patients, the distribution of tonsil 9G4+B-cells is similar to healthy individuals, but only limited phenotypic analyses have examined 9G4+B-cells in peripheral blood. The aim of this work was to perform an extensive phenotype characterization of 9G4+B-cell sub-populations in peripheral blood in RA patients and healthy controls (HC).
Methods: Blood samples were collected from established RA patients and age and sex-matched HC. Peripheral blood mononuclear cells were isolated and 9G4+B-cells (gated in CD19+B-cells) were characterized by flow cytometry.
Results: The frequency of total 9G4+B-cells in circulation was similar between RA patients and HC. RA patients had a higher frequency of 9G4+IgD-CD27- B-cells and 9G4+naïve B-cells (IgD+38++) than HC, but had significantly lower levels of 9G4+transitional B-cells (IgD+CD38+++) and 9G4+plasmablasts (IgD--CD27+++CD38+++) when compared to HC. BAFF-R expression was significantly increased in 9G4+B-cells from RA patients compared to HC. The levels of 9G4+CD5+ B-cells were significantly decreased in RA patients, but no other significant differences were found in 9G4+B-cells expressing CD24, IgM and CXCR5.
Conclusions: RA patients have alterations in the frequency of 9G4+B-cell subpopulations when compared to HC. The significant reduction in the frequency of 9G4+transitional and 9G4+CD5+ B-cells and the increased levels of 9G4+naïve B-cells suggests a more rapid maturation of 9G4+B-cells in RA patients following entry into the peripheral pool. This could be influenced by the antigen specificity of the B-cell receptor. Furthermore, the increased BAFF-R expression by 9G4+B-cells observed in RA patients might support an increased survival mechanism for these already inherently autoreactive B-cells.

09

MIR-146A DEFICIENCY IN LY6C^{HIGH} MONOCYTES CONTRIBUTES TO PATHOGENIC BONE LOSS DURING INFLAMMATORY ARTHRITIS

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Introduction: Monocytes represent a prototypic cell type when investigating the interplay between immune and skeletal systems as they can give rise to different cell types including dendritic cells, macrophages and osteoclasts (OC), which play key roles in immunity and bone homeostasis. Circulating monocytes consist of at least two main functional subsets, Ly6C^{high} and Ly6C^{low} monocytes. It has been suggested that OC might develop preferentially from the Ly6C^{high} monocyte subset, which excessive and prolonged activation is a hallmark of many inflammatory diseases. Among key molecular rheostats of cell fate, micro(mi)RNAs are a class of regulatory RNAs that control basic biological functions and orchestrate inflammatory responses. Few

miRNAs have been involved in osteoclastogenesis. The present study aimed at investigating the role of miRNAs in osteoclastogenesis in the context of monocyte subsets, under steady state and inflammatory conditions.
Methods and Results: Genome-wide miRNA expression study identified miRNAs and putative targeted pathways that are differentially expressed between $Ly6C^{high}$ and $LyC6^{low}$ FACS sorted mouse monocytes, and common to their human counter parts $CD14^{+}CD16^{-}$ and $CD14^{dim}CD16^{+}$ monocytes, respectively. Among these, miR-146a showed lower expression in $Ly6C^{high}$ monocytes when compared to $Ly6C^{low}$ monocytes. Under inflammatory arthritis conditions, expression of miR-146a in $Ly6C^{high}$ monocytes was down regulated as compared to healthy controls. Using mouse deficient for miR-146a, we showed that deficiency for miR-146a increased OC differentiation and bone erosion both in vitro and in vivo, respectively. Using a liposomal formulation able to deliver RNAi triggers to $Ly6C^{high}$ monocytes upon intravenous injection, we showed that enforced expression of miR-146a both in vitro and in vivo led to decreased TRAP positive cells, reduced bone loss and was associated with decreased RelB expression.
Conclusion: Overall, our results show that specific over-expression of miR-146a in $Ly6C^{high}$ monocytes altered OC differentiation and decreased bone erosion in inflammatory arthritis. These data suggest a novel role for miR-146a in controlling osteoclast fate of $Ly6C^{high}$ monocytes and that reduced miR-146a expression in $Ly6C^{high}$ monocytes under arthritic conditions contributes to pathogenic bone loss. Finally, delivery of miR-146a mimics to $Ly6C^{high}$ monocytes may offer valuable therapeutic potential to interfere with pathological bone loss.

O10

HUMAN MAST CELLS ENGULF AND STORE EXOGENOUS IL-17A

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Background: IL-17A plays an important role in pathophysiology of spondyloarthritis, as IL-17 blocking therapy was effective in the first phase II trial. Analysis of inflamed synovium revealed an abundant presence of IL-17A-positive mast cells. This population was specifically increased in patients with SpA compared to RA and was not modulated by TNF-blocking therapy, suggesting that IL-17A-positive mast cells are specific for the pathophysiology of SpA and that they act upstream of general inflammation.
Objectives: As mast cells are not known to produce IL-17A in mice, we aimed to investigate the mechanism of IL-17A expression by human mast cells.
Methods: mRNA and protein expression was assessed ex vivo and after PMA/ionomycine stimulation in primary human mast cells from tonsil and synovium. Internalization of exogenous IL-17A was assessed by Western blot, image stream, live imaging and confocal microscopy. Release of IL-17A was assessed by ELISA.
Results: Immunostaining and western blot analysis confirmed the presence of IL-17A protein in primary human mast cells. However, in contrast to T cells, mast cells did not express RORC protein, the indispensable transcriptional factor controlling IL-17A expression. Accordingly, *IL17A*, *IL17F*, and *RORC* gene expression was readily detectable in sorted T lymphocytes but not in mast cells, even after stimulation. Given the discrepancy between the presence of IL-17A protein and absence of its transcriptional machinery, we investigated the uptake of recombinant IL-17A. Western blot and imaging studies indicated that both primary mast cells and the LAD2 mast cell line engulf and store exogenous IL-17A. This uptake can be blocked by inhibiting receptor-mediated endocytosis. Engulfed IL--17A can be released back to the milieu upon external stimuli.
Conclusions: Human mast cells do not produce IL--17A but engulf and store exogenous IL-17A and possess ability to release the IL-17A back to the milieu.

O11

B-CELL MARKERS EXPRESSION IS AFFECTED BY TNF-INHIBITORS AND TOCILIZUMAB TREATMENT IN RHEUMATOID ARTHRITIS

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Introduction: The use of immunosuppressive drugs such as TNF-inhibitors and/or Il-6 receptor antagonist, tocilizumab, in rheumatoid arthritis (RA) might affect the circulating numbers of B-cells and their activation state. The main goal of this work was to study the effect of TNF-inhibitors and tocilizumab on B-cell phenotype and gene expression before and after treatment.
Methods: Blood samples were collected from established RA patients treated with TNF-inhibitors (n=10) and tocilizumab (n=9) before and after treatment, and from healthy donors (n=15). B-cell subpopulations were characterized by flow cytometry and B-cell gene expression was analyzed by real-time PCR on isolated B-cells.
Results: The frequency of circulating B-cell subpopulations was similar between controls and established RA patients irrespective of treatment. The mean fluorescence intensity (MFI) of TACI, CXCR5 and HLA-DR expressed on CD19+B-cells was significantly increased in TNF-treated patients when compared to controls. A significant decrease of CD86 and increase of CD95 MFI was observed in CD19+B-cells after TNF treatment. In tocilizumab-treated patients, HLA-DR and TLR9 MFI were significantly increased in CD19+B-cells when compared to controls. No significant differences were observed in BAFF-R, BCMA, IgM and CD69 MFI in both groups of patients compared to controls. BAFF-R, Bcl-2, β2-microglobulin, FcγR2A, TLR7 and TLR10 gene expression were significantly increased after treatment with TNF and/or tocilizumab in comparison with controls, but no significant differences were observed when comparing baseline and follow-ups.
Conclusions: In RA, the use of TNF-inhibitors and/or tocilizumab treatment affects B-cell phenotype in circulation, but the effect of these drugs on B-cell gene expression is less evident. The decreased levels of CD86 expression and increased CD95 MFI on B-cells after TNF treatment support an inhibition of B-cell activation. Our results suggest that TNF-inhibitors and tocilizumab help to reduce B-cell infiltration in the joints and these activated B-cells express higher HLA-DR and CXCR5 levels in circulation.

012

FIBROBLAST-LIKE SYNOVIOCYTES (FLS)-SPECIFIC APTAMER FACILITATES THE CONJUGATED-TRIPTOLIDE SELECTIVELY TARGETING RHEUMATIC JOINTS WITH LESS EXPOSURE TO LIVER IN RODENTS

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Introduction/Aim: Triptolide (TP) is regarded as an anti-RA candidate with multi-effects including modulating the activities of FLS, key effectors in RA pathology. However, severe hepatotoxicity due to nonspecific exposure hinders its usage. Aptamers are oligonucleotides which can act as targeting ligand to bring drugs to target cells. Thus, it is desirable to test the hypothesis that FLS-specific aptamer could facilitate the conjugated-triptolide selectively targeting rheumatic joints with less exposure to liver.
Methods: Targeting aptamers were obtained by Cell--SELEX with target cells (FLS from CIA mice) and negative cells (FLS, liver cells and PBMC from normal mice) selection. The pathologic FLS-targeting aptamer was conjugated to TP by a pH-sensitive linker that can be hydrolyzed in the acidic environment within rheumatic joints. The *in vitro* pH-stability, cell-selectivity, and *in vivo* tissue distribution of the conjugate was examined by HPLC.
Results: We obtained an aptamer with high selectivity toward pathologic FLS. TP was successfully conjugated with the aptamer by the pH-sensitive linker. The conjugate was stable at physiological pH but ruptured in acidic environment that mimic the pathologic joints. The conjugate was up-taken more by pathologic FLS with less accumulation in normal cells *in vitro*. In CIA mice treated with the conjugate, TP was found more in rheumatic joints but less in liver when compared to the treatment with free TP.
Conclusion: The FLS-specific aptamer could facilitate TP selectively targeting the rheumatic joints with less exposure to liver.

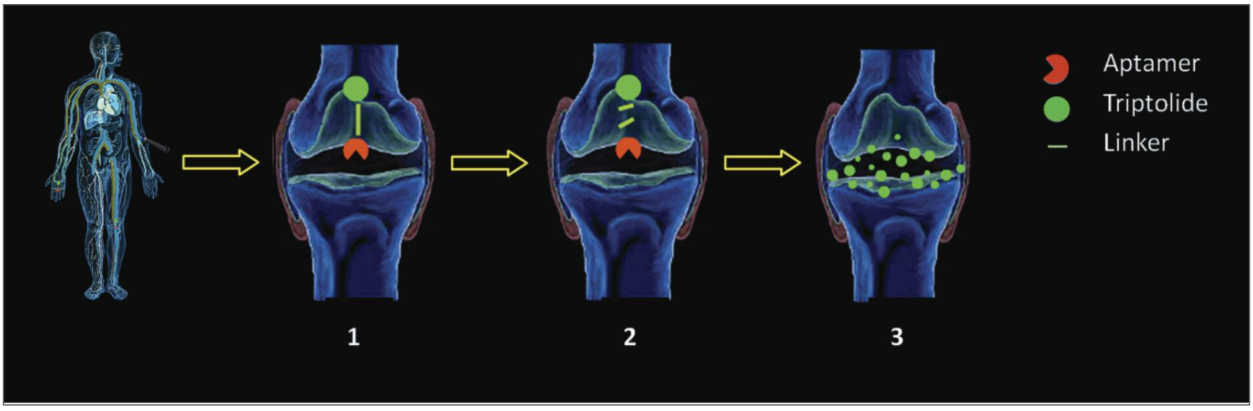

FIGURE.

ACKNOWLEDGEMENT
This study is supported in part by the projects from Ministry of Sciences and Technology of China (No. 2013ZX09301307) and China Academy of Chinese Medical Sciences (No. Z0252 and Z0293).