monocytes with a panel of TLR ligands demonstrated strong TIMP-1 and IL-6 production following triggering with TLR8 agonists (ssRNA). TLR8-mediated TIMP-1 production was reduced in monocytes from a patient with a genetic TLR signalling defect or HC monocytes cultured with MyD88 inhibitory peptide. Furthermore, matrix assay of TLR8 stimulated monocytes also confirmed functional TIMP-1 secretion, as matrix metalloproteinase-1 activity was significantly inhibited.

Conclusions This study indicates a potential link between SSc serum factors and TLR signalling resulting in excessive TIMP-1 secretion by circulating monocytes from SSc patients.

A2.21 TOLL-LIKE RECEPTOR DEPENDENT AUTOANTIGENS AND VESICLES FROM P. GINGIVALIS IN ANIMAL MODELS OF RA TO MODULATE COLLAGEN AND COLLAGEN ANTIBODY INDUCED ARTHRITIS

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Background and Objectives A variety of animal models suggest that TLR signalling is important in the pathogenesis of RA and the generation of specific autoantibodies. This study was conducted with sera from patients with rheumatoid arthritis, as well as with arthritis animal models to identify identical autoantigens dependent on TLR7 and 9 in human and animal models for disease modifying use. Moreover TLR2 and TLR4 modulating bacterial vesicles from P. gingivalis containing PAD (Peptidyl-Arginine Deiminase) which is involved in citrullination was used to study the TLR2/4 in arthritis.

Materials and Methods Using protein philtre technology (28000 human protein philtre) the autoantigen profile of RA patients, mouse collagen and zymosan induced arthritis, as well as collagen and pristan induced arthritis in rats and TLR7, TLR9 deficient double-deficient and MyoD88 and Tir8 deficient mice of the MRL-lpr/lpr background were obtained. Cationic liposomes transferring siRNAs, bacterial vesicles, lipomannan and LPS were used for the validation of their potential as therapeutic target in collagen or collagen antibody induced arthritis (CAIA).

Results We found 18 identical proteins targeted in human and animal situations of arthritis. These data identify mRNA binding hnRNPs proteins which are part of P bodies, stress granules and components of messenger RNA stability complex as well as CRP binding proteins as target molecules in mice, rats and humans with RA. Moreover, we found MyoD88 independent autoantigens which are not expressed in the thymus or proteins such as high mobility group box proteins 1 and 2 which are MyoD88 independent sensors of nucleic-acid-mediated innate immune responses. Systemic administration of siRNAs with cationic liposomes inhibiting expression of Toll dependent autoantigens overexpressed and targeted by autoantibodies in the human and mouse synovial tissue were used for the validation of their potential to inhibit collagen induced arthritis in C57BL/6J mice. Moreover P. gingivalis vesicles containing the PAD induce a mild inflammatory response in the CAIA model of arthritis. P. gingivalis LPS and lipomannan treated animals show a 80% reduction of athrithis score compared to E coli LPS in a C57BL/6J CAIA model.

Conclusions Systemic blocking of common RNA or DNA binding proteins overexpressed in synovial target tissue appears to modify arthritis. *P. gingivalis* vesicles evolved the ability to intercept and undermine a subset of TLR2/4 signalling events for corrupting innate immunity and modulate RA.

A2.22 TYROSINE PHOSPHORYLATION PATHWAYS IN MYELOID CELL-MEDIATED INFLAMMATORY DISEASES

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Background Tyrosine kinases are major therapeutic targets in cancer but their role in immune-mediated disease pathogenesis is less understood.

Methods Here we tested the role of tyrosine kinases and tyrosine kinase substrates in two autoantibody-mediated disease models, the K/BxN serum-transfer arthritis and anti-collagen VII autoantibody-induced blistering skin diseases.

Results Mice genetically deficient of the Syk tyrosine kinase in the hematopoietic compartment were completely protected from autoantibody-induced arthritis and blistering skin disease. Mice lacking three myeloid-specific tyrosine kinases (Hck, Fgr and Lyn) or the tyrosine kinase substrate PLCγ2 were also protected from autoantibody-induced diseases. In vitro, Src-family kinases were required for Syk activation by immune complexes and both were further required for activation of PLCγ2. The Src-family–Syk–PLCγ2 pathway mediated cytokine production by myeloid cells but not neutrophil or monocyte migration per se. Lineage-specific analyses revealed that during autoantibody-induced arhritis, this signalling pathway was required in myeloid cells (particular neutrophils) but not in mast cells or platelets. Finally, Src-family kinases were also required for activation of myeloid cells by monosodium urite crystals and their deficiency attenuated monosdium urate crystal-induced arthritis, indicating that the role of this signalling pathway is not restricted to autoantibody-mediated disease processes.

Conclusions Taken together, the Src-family–Syk–PLCγ2 pathway is an important component of both autoantibody-mediated and autoantibody-independent inflammatory disease processes.

A2.23 IMPAIRED NATURAL KILLER CELL FUNCTION IN DOCK8 DEFICIENCY

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Introduction DOCK8 mutations are responsible for a rare autosomal recessive immunodeficiency syndrome associated with severe cutaneous viral infections, elevated IgE levels, environmental allergies, autoimmunity, and malignancy. DOCK8 activates CDC42, which is important for cell signalling and actin reorganisation. Natural killer cells play a vital role in tumour surveillance and defence against virally infected cells. NK cell function relies on the accumulation of actin at the NK cell immunologic synapse formed with target cells. Although abnormalities in T and B cell function have been described in DOCK8-deficient patients, the role of NK cells in this disease is poorly understood.

Objectives/Aims Given the susceptibility to severe cutaneous viral infection and malignancy, we hypothesised there was a substantive defect in NK cell function in patients with DOCK8 deficiency.
Methods 10 patients with genetically confirmed DOCK8 deficiency as well as NK cell lines with stably reduced DOCK8 expression were evaluated experimentally using in vitro NK cell cytotoxicity, F-actin content, and confocal immunofluorescence microscopy assays.
Results DOCK8-deficient patients and cell lines all had decreased NK cell cytotoxicity and function could not be restored after IL-2 stimulation. Importantly, DOCK8 deficiency did not affect NK cell F-actin content, but impaired F-actin accumulation at the lytic immunological synapse.
Conclusions DOCK8 deficiency results in severely deficient NK cell function owing to an inability to form a mature lytic immunological synapse via focal F-actin accumulation. This defect may underlie important and previously perplexing attributes of the DOCK8 deficiency clinical syndrome including the unusual susceptibility to viral infection.

A2.24 IL23/TH17-MEDIATED REGULATION OF ANTIBODY GLYCOSYLATION CONTROLS AUTOIMMUNE-INDUCED ARTHRITIS

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Background and Objectives Both rheumatoid arthritis (RA) and the murine models of collagen-induced arthritis (CIA) and of K/BxN arthritis are characterised by an initial break in self-tolerance, the appearance of specific autoantibodies and an autoantibody-mediated effector phase resulting in chronic inflammation and joint destruction. The IL23-dependent Th17 T-cell response has been identified as a major driving force during the pathogenesis of these disorders. The exact contribution of the IL23/Th17 axis to autoimmune-triggered inflammation, however, has remained incompletely understood. In this study, we aimed to further elucidate the role of IL23 and Th17 T-cells during murine autoimmune arthritis.
Materials and Methods To study and dissect the contribution of Th17 T cells to the initiation and effector phase of autoimmune arthritis, we performed the CIA as well as the K/BxN serum transfer model of arthritis in both wild-type (WT) mice and in mice lacking the IL23-specific subunit p19. Subsequently we determined the clinical course of disease as well as the serum levels, the avidity and the glycosylation pattern of antibodies in the sera of the respective mice.
Results While IL23$^{-/-}$ mice, which lack functional Th17 T-cells, developed a full-blown arthritis after passive transfer of autoantibodies in the K/BxN model, these mice were resistant to collagen-induced arthritis. These data indicated that the IL-23/Th17 axis is dispensable during the autoantibody-mediated effector phase of arthritis, whereas it is crucially involved in mounting an autoimmune response during CIA. Despite being protected from CIA, IL23$^{-/-}$ mice displayed regular levels of anti-collagen antibodies, which also showed a regular avidity. Likewise, we observed no difference in the IgG subclasses between the two genotypes. Analysis of the glycosylation pattern of antibodies in the sera of WT and IL23$^{-/-}$ mice, however, revealed major differences in the content of sialic-acid and fucose residues at the Fc part of the IgGs resulting in an anti-inflammatory IgG profile in the sera of IL23$^{-/-}$ mice. The changes in the IgG glycosylation, in turn, correlated with changes in the expression pattern of glycosyltransferases in plasmablasts and plasmacells of WT and IL23-deficient mice.
Conclusions Together, these data show that the IL23/Th17 axis controls the degree of antibody glycosylation and, in turn, indicate that this regulation of the glycosylation of autoantibodies is a critical step in the pathogenesis of Th17-mediated autoimmune diseases such as RA.

3. T cells – activation and regulation

A3.1 1.25(OH)$_2$D$_3$ INHIBITS TH17 POLARISATION AND RORγt EXPRESSION THROUGH GATA3-DEPENDENT AND -INDEPENDENT MECHANISMS

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Background and Objectives Vitamin D has suppressive effects on autoimmune diseases, such as rheumatoid arthritis (RA). Regulation of Th17 cell activity is an important mechanism by which vitamin D exerts these effects. Aside from inhibiting Th17 cytokines and the Th17 transcription factor RORγt, vitamin D induces IL-4 and GATA3. Since GATA3 over-expression inhibits experimental Th17-mediated autoimmunity, we studied the contribution of GATA3 in vitamin D-mediated suppression of Th17 polarisation.
Methods Therefore CD4$^+$ T cells were sorted from patients with early RA, naïve DBA-1 mice, DBA-1 mice immunised with collagen type II (CII) or naïve CD2-GATA3 transgenic mice and cultured under T helper cell polarising conditions with or without 1.25(OH)$_2$D$_3$, the active form of vitamin D.
Results 1.25(OH)$_2$D$_3$ inhibits Th17 polarisation in CD4+ cells from both non-immunised and CII-immunised mice, while up-regulating IL-4 and GATA3 expression. In these cultures, IL-4 inhibition partly reversed the vitamin D-mediated inhibition of Th17 polarisation. Moreover, GATA3 over-expression reduces Th17 differentiation to a lower level than 1.25(OH)$_2$D$_3$. Interestingly, combining GATA3 over-expression and 1.25(OH)$_2$D$_3$ treatment reduced IL-17A and RORγt expression even further. Furthermore, gene-expression analysis showed that NFAT-C2, which is involved in IL-17A production, was down-regulated by 1.25(OH)$_2$D$_3$. In addition, in T cells from patients with RA, 1.25(OH)$_2$D$_3$ inhibited Th17 cytokine and RORγt expression and induced IL-4 and GATA3 expression.
Conclusions These data show that vitamin D-mediated regulation of Th17 polarisation occurs through GATA3-dependent mechanisms, including direct effects on RORγt expression and IL-4-mediated inhibition of Th17 polarisation. Moreover, GATA3-independent mechanisms are involved that may include modulation of NFAT-C2 expression.

A3.2 A CD4+ T-CELL GENE EXPRESSION SIGNATURE PREDICTS DRUG SURVIVAL ON METHOTREXATE MONOTHERAPY IN EARLY RHEUMATOID ARTHRITIS

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Background/Purpose The mechanism of action of methotrexate (MTX) in the management of rheumatoid arthritis (RA) remains