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)₂D₃ INHIBITS TH17 POLARISATION AND ROR γ t 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 ROR γ t, 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)₂D₃, the active form of vitamin D.

Results 1.25(OH)₂D₃ 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)₂D₃. Interestingly, combining GATA3 over-expression and 1.25(OH)₂D₃ treatment reduced IL-17A and ROR γ t 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)₂D₃. In addition, in T cells from patients with RA, 1.25(OH)₂D₃ inhibited Th17 cytokine and ROR γ t 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 ROR γ t 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

incompletely understood. Making use of an existing dataset, we investigated the CD4+ T-cell transcriptome in early RA patients, seeking biomarkers for drug survival on MTX monotherapy and associated pharmacological insights.

Methods In previous work [1] RNA was rapidly extracted from peripheral blood CD4+ T-cells of 173 early arthritis clinic attendees, at which time patients had been symptomatic for a median of 12 weeks, and were naïve to immunomodulatory treatments. Transcriptional profiling was undertaken using Illumina WG6v3 BeadChip oligonucleotide array technology. For this study a sub-cohort of RA patients was retrospectively identified, whose initial treatment with MTX monotherapy was continued for as long as deemed successful between patient and consulting rheumatologist. Intra-muscular steroid bolus administration (but not oral steroid therapy) was permitted during the study at the discretion of the consulting rheumatologist. MTX monotherapy survival after ≥ 1 year follow-up (median 28 months) was used as a surrogate outcome for efficacy, and bioinformatics analysis was performed using GeneSpring XI (Agilent).

Results Amongst 31 eligible patients, 19 (61%) remained on MTX monotherapy at the end of follow-up, but the treatment strategy was unsuccessful (and required modification) for the remaining 12 (39%). Baseline characteristics and final methotrexate doses were comparable between the two groups. 133 CD4+ T-cell transcripts were identified as being differentially expressed between comparator groups at baseline (>1.2 fold-change; $p < 0.05$) and a metric derived from their normalised expression values demonstrated a promising discriminatory utility with respect to MTX monotherapy survival (area under ROC curve 0.91). Functional analysis identified an over-representation of genes involved in apoptosis (11/133 genes; hypergeometric $p = 0.000045$).

Conclusions Although limited by its reliance on a surrogate efficacy outcome, our pilot study has identified potential transcriptional biomarkers for drug survival on MTX monotherapy amongst early RA patients. Alongside their potential clinical applicability, they suggest that this treatment's efficacy may depend on its ability to regulate CD4+ T-cell survival. Validation amongst a clinically well-characterised, independent early RA cohort is now on-going.

Reference

1. Pratt AG *et al*, *ARD* 2012.

A3.3 AN IMMUNOLOGICAL WINDOW OF OPPORTUNITY DEFINES THE ABILITY OF EARLY RA PATIENT TO ACHIEVE REMISSION WITH FIRST ANTI-RHEUMATIC TREATMENT

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Background and Objectives The therapeutic goal for patients with rheumatoid arthritis (RA) is clinical remission. This is best achieved by early diagnosis followed by appropriate therapeutic intervention. RA is associated with dys-regulation of T-cell subsets early in the disease with naïve cells and regulatory T-cell losses and acquisition of abnormal subset in relation with inflammation (IRC). Our aim was to test the hypothesis that T-cell subset quantification can predict the achievement of clinical remission in patients with early RA.

Materials and Methods T-cell subsets (naïve, Treg and IRC) were quantified using flow cytometry in 108 DMARDs-naïve, early RA patients (<24 months) symptom duration commencing methotrexate MTX or MTX + anti-tumour necrosis factor agents (anti-TNF) and in 105 healthy controls (HC). The primary outcome assessed was remission (DAS28 < 2.6). The pilot analysis was performed on frozen PBMC obtained from 38 RA patients and 35 HC. These results were validated using fresh blood samples on a cohort of 70 RA patients and 70 HC.

Results In the pilot study, T-cell subset analysis in early RA confirmed immune dysregulation compared to HC with reduced

frequency of naïve CD4+ T-cells and Treg and increased IRC (all $p < 0.001$) compared to HC. Naïve T-cell above median was associated with remission ($p = 0.001$). In the validation study, 50 patients were treated with MTX and showed the same relationship with naïve cell frequency above median being associated with remission ($p = 0.011$). Individual analysis on each patient's naïve cell frequency deviation from expected (using 70 HC) demonstrated that "normal" naïve cell frequency (observed in 30 patients) was associated with remission whereas reduced naïve cell frequency was more frequently observed in patients with poor response to MTX ($p = 0.03$). Patients with poor immunological status were not prevented to achieve remission when treated with MTX + anti-TNF ($n = 20$ including 10 patients with normal and 10 with reduced naïve cells) raising the rate of remission from 20% in the MTX group ($n = 4$ of the 20 patients with reduced naïve cells at baseline) to 60% in the MTX + anti-TNF group ($n = 6$ of the 10 patients).

Conclusions These data show that baseline naïve T-cell subset analysis has a value in predicting early RA MTX treatment outcome. Immunological analysis could be used in conjunction with clinical/serological features to predict response to MTX and select the most appropriate therapy at disease presentation.

A3.4 CD3+CD4-CD8- DOUBLE NEGATIVE TH17 CELLS: NEW INSIGHTS IN THE PATHOGENESIS OF PRIMARY SJÖGREN'S SYNDROME

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Background and Objectives IL-17 axis is widely recognised to be involved in the pathogenesis of autoimmune disorders. Besides conventional CD4+ Th17 cells, a small IL-17 producing T-cell population, that lacks of both CD4 and CD8 molecules, defined as double negative (DN) was recently found to be expanded in the peripheral blood (PB) and to accumulate in the kidney in patients with lupus nephritis. Since IL-17 production is enhanced in minor salivary gland (MSG) infiltrates of patients with primary Sjögren's syndrome (pSS), we sought to investigate whether DN T cells may be involved in pSS pathogenesis.

Materials and Methods Thirty patients with pSS and 16 normal controls (NC) were studied. PBMCs were separated by density gradient and phenotypic characterisation was performed by flow cytometry on both freshly isolated cells and after culture (24, 48, 72 and 96 hours). Total PBMCs were cultured in anti-CD3 coated plates in presence or absence of dexamethasone (Dex) at different concentrations. In selected experiments, real time PCR at the same time-points was performed. The study of pSS-MSGs was performed by immunofluorescence.

Results Total circulating DN T cells were increased in pSS compared to NC. NC and pSS freshly isolated DN T cells expressed ROR γ t, activation markers (CD25, CD69, HLA-DR) and produced consistent amounts of IL-17. Despite IL-6/TGF β ratio became abnormal in pSS patients after 72 hour-culture, Dex was able to down-regulate IL-17 in vitro production in NC and pSS CD4+Th17 cells and in NC DN T cells from this time-point on. Surprisingly, IL-17 production by pSS-DN T cells was not affected at all by Dex at any time-point. Dex could also reduce the expression of activation markers on CD4+ cells, but not in pSS and NC-DN T cells. Among DN T cells, those expressing α BTCTCR were expanded in patients with active pSS compared to those with inactive pSS. DN T cells were present in pSS-MSG infiltrate.

Conclusions To our knowledge, this is the first study identifying and characterising DN T cells in pSS. It shows that DN T cells are