patients. CD4+ T cells, derived from PBMCs of healthy donors, were used to obtain T-cell clones. CD4+ T-cell clones were classified on the basis of their ability to produce IFN-γ and/or IL-17. T cell clones were polyclonally stimulated with anti-CD3/CD28. Cytokine expression was assessed with RT-PCR and production in supernatants by ELISA.

Results As previously described, IL-17A and TNF-α had a synergistic effect for both cells. Moreover, a stronger induction for IL-6 production was seen for synoviocytes compared to EC (1005 ± 22 pg/ml versus 250 ± 15 ng/ml respectively). Supernatants from inactivated Th1 or Th17 clones had no effect on EC. Regarding synoviocytes, supernatants from inactivated clones induced IL-6 and IL-8 mRNA with a stronger effect for Th17 cells (1000 fold versus 100 fold compared to resting synoviocytes, p = 0.045). While activated Th1 supernatants had a strong effect on EC and increased the expression of IL-6, IL-8, E-selectin, and tissue factor mRNA (98.12, 89 fold respectively, compare to control), Th17 supernatants had no effect. Activated Th1 and Th17 supernatants had the same effect on synoviocytes (254 and 754 fold compared to control). Th1 cells were more potent inducers of IL-6, IL-8 and tissue factor mRNA in EC than Th17 which had no significant effect (5, 12.5, 7.5 fold respectively compared to control). Th17 cells were most effective to stimulate IL-6 and IL-8 mRNA expression in synoviocytes compared to Th1 (100 fold versus 24 for IL-6 mRNA, p = 0.027). Anti-IL-17 antibody reduced IL-6 production from 20.0 ± 4.5 ng/mL to 13.2 ± 3.9 ng/ml (p = 0.032) in synoviocytes cultured with Th17 clones.

Conclusions While Th1 cells were able to induce inflammatory cytokine expression in EC and synoviocytes, Th17 cells were only active to promote inflammation in inflammatory cells such as synoviocytes. The effect of Th17 cells appears to depend of IL-17 and the type of stromal cells.

A3.8 EXTRATHYMIC AUTOIMMUNE REGULATOR (AIRE) EXPRESSION IN RHEUMATOID ARTHRITIS

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Background AIRE is a transcription factor that is involved in the negative selection of self-reactive thymocytes in the thymus. Recently, AIRE protein has also been detected in peripheral lymphoid organs, predominantly in dendritic cells (DC). In these peripheral sites, AIRE was found to regulate the expression of a group of tissue-specific antigens that is distinct from those expressed in the thymus, suggesting that peripheral AIRE may play a complementary role in tolerance induction. It is currently unknown whether AIRE may play a role in inflamed tissues, such as rheumatoid arthritis (RA) synovial tissue (ST).

Objective To document and further characterise extrathymic AIRE expressing cells in ST and paired peripheral blood (PB) mononuclear cells (MCs) as well synovial fluid (SF) MCs of RA patients.

Methods ST was obtained via mini-arthroscopy from inflamed joints of RA or undifferentiated arthritis (UA) patients. Expression of AIRE was evaluated using IHC and IF microscopy. AIRE expression was also investigated in PB and SF DC using flow cytometry.

Results AIRE expressing cells were detected in 80% of analysed RA ST and in contrast only in 25% of UA ST. Further characterisation using double-immunofluorescence microscopy revealed that these cells were predominantly CD1c+ myeloid (m)DC. Interestingly, a significantly higher percentage of CD1c+ mDC in RA SF expressed AIRE (55 ± 5%) compared to RA PB (20 ± 3%) and healthy PB (19.7 ± 2%).

Conclusions Extrathymic AIRE expressing CD1c+ mDCs are present in RA ST and RA SF, suggesting a role in synovial inflammation. Extrathymic AIRE expression in RA synovial inflammation may be an attempt to control inflammation through the induction of peripheral tolerance to antigens involved in the perpetuation of the chronic inflammatory response.

A3.9 IL-17RA SIGNALLING IS ESSENTIAL FOR COLLAGEN INDUCED ARTHRITIS DEVELOPMENT

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Introduction IL-17A plays an important role in collagen-induced arthritis (CIA). It signals through the IL-17 receptor (IL-17R) A and C heterodimer. The IL-17RA appears to be a common receptor subunit for several IL-17 cytokine family members, including IL-17A, IL-17C and IL-17F. Lack of IL-17RA signalling may therefore have a broader effect than lack of IL-17A alone. We therefore aim to determine the role of IL-17RA signalling in arthritis.

Methods Disease incidence and severity were scored in wild type (positive control), IL-17RA deficient and IL-23p19 deficient (negative control) mice in CIA. T helper cell profiles and humoral immune responses were analysed by flow cytometry and immuno-histochemistry. Serum auto-reactive IgG antibodies were measured by ELISA. Pathogenicity of T cells and total splenocytes was determined in a functional assay in vitro.

Results As expected, wild type mice developed CIA from day 21 after the first immunisation. IL-23p19 deficient mice did not develop arthritis. Interestingly, IL-17RA deficient mice were completely protected against CIA, even after a third immunisation. This is in contrast to IL-17A deficient mice, of which 20% is still susceptible to CIA. T cells in IL-17RA deficient mice display a Th2-like phenotype in CIA with higher proportions of IL-4 producing CD4 T cells. This population is distinct from IL-17A producing T cells. The shift in T cell phenotype induces a less inflammatory B cell response with fewer plasma cells in the spleen and lower pathogenic IgG2a antibody production in favour of IgG1 production. In a functional assay, both isolated CD4+ T cells and total splenocytes of IL-17RA deficient mice were less capable of inducing pro-inflammatory IL-6 production by normal, IL-17RA expressing synovial fibroblasts in CIA in vitro.

Conclusions Here we show that lack of IL-17RA signalling prevents auto-immune inflammation of the joint. In addition, T helper cells shift to a Th2 like phenotype characterised by IL-4 production. T cells and splenocytes of these mice are less pathogenic, leading to lower pathogenic IgG2a antibody levels in serum. This is in contrast with IL-17A deficient mice and suggests that other factors involved in CD4+ T cell differentiation and pathogenicity can signal through the IL-17RA.

A3.10 IL-6 RECEPTOR BLOCKADE ENHANCES CD39+ REGULATORY T-CELL DEVELOPMENT IN RHEUMATOID ARTHRITIS AND IN EXPERIMENTAL ARTHRITIS

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Background and Objectives Studies have demonstrated the clinical efficacy of tocilizumab, a humanised anti-IL-6 receptor (R) antibody (Ab), in patients with rheumatoid arthritis (RA). The rational

for blocking IL-6 in this disease mainly lays on the pro-inflammatory role of this cytokine in the disease. However, only few works have studied the consequences of anti-IL-6R treatment on Tregs cells and mainly focuses on their frequency. Our objective was to elucidate anti-IL-6R mode of action on Tregs in RA patients treated with tocilizumab and in a RA model.

Methods Mice with collagen-induced arthritis (CIA) were treated at day 0 by MR16–1 (a rat anti-mouse IL-6 receptor monoclonal Ab provided by Chugai Pharmaceutical Co. LTD, Japan) and the evolution of $CD4^+$ $FoxP3^+$ Tregs during arthritis course was assessed at key time points (day 8–18–28 and 42 after CIA induction) by studying their number, frequency and phenotype (expression of GITR, ICOS, Helios, CD62L, CTLA-4 and CD39) in lymph nodes (LN), thymus and spleen by flow cytometry. Numerical analysis of Th17 and Th1 cells was also performed by flow cytometry. Twenty patients with severe and active RA were recruited and treated with 8 mg/kg of tocilizumab monthly. Peripheral blood was recovered at day 0, as well as 1 and 3 month, and Th17and Tregs were analysed by flow cytometry.

Results Clinical and histological evaluation of arthritides in mice treated with anti-mouse IL-6R mAb showed, as expected, a less severe disease as compared to control Ig treated mice. Th17 frequency was unchanged, but Tregs frequency was enhanced in the LN of MR16–1 treated mice. In the thymus, we observed an enhanced frequency of Tregs $CD4^+CD8^-FoxP3^+$. Tregs phenotype was also modified in treated mice, with an increased frequency of $CD39^+$ Tregs (LN and spleen), suggesting an enhanced ATP hydrolysis immunosuppressive activity of Tregs. In RA patients, Th17 frequencies were not modified by tocilizumab therapy and did not differ between responders and non-responders. Interestingly, $CD39^+$ Treg cell among $CD4^+$ cells frequencies were significantly higher in responders than in non-responders after 3 months of tocilizumab therapy.

Conclusions Tregs, but not Th17, are modified by anti-IL-6R treatment in both CIA and RA. These results support a beneficial effect in RA of treatments responsible for $CD39^+$ Tregs enhancement and emphasise the relevance of the monitoring cell populations after cytokine blockade used to treat arthritis.

A3.11 IMMUNOSUPPRESSIVE EFFECTS OF GLUCOCORTICOIDS AND REGULATORY T CELLS ON CD28NULL T CELLS IN VITRO

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Background and Objectives CD28null T cells are terminally differentiated T cells lacking CD28 co-receptor. These cells display properties of proinflammatory killer cells and are suggested to be resistant to apoptosis in vivo. Frequencies of CD28null T cells are increased in various chronic, inflammatory diseases. CD28null T cells dominate both in the affected muscle and peripheral blood of patients with idiopathic inflammatory myopathies (myositis), suggesting a role these cells in disease mechanism and muscle pathology. Recently, it was found in our lab that after conventional glucocorticoid treatment, the relative number of regulatory T cells (Tregs) was unchanged or decreased, while the CD28null T cell proportion was mainly increased in muscle tissue of myositis patients. This lead to our working hypothesis that CD28null T cells are resistant to immunosuppression mediated by glucocorticoids in the setting of myositis. Such resistance could also be against Tregs mediated immunosuppression due to distinct phenotype of CD28null T cells. The aim of this study was to evaluate the immunosuppressive effects of glucocorticoids and Tregs on CD28null T cells in an in vitro system.

Method Peripheral blood mononuclear cells (PBMCs) were obtained from 3 healthy individuals using Ficoll separation. CD3+CD4+ CD25++(high) cells were sorted as Tregs by flow cytometry. For glucocorticoid or Tregs mediated T cell suppression assays, PBMCs were stimulated with plate bound α-CD3 antibody in presence of 4uM glucocorticoid (methyl prednisolone sodium succinate) or optimal proportion of Tregs. Up-regulation of the early activation marker CD69 was measured by flow cytometry. Suppression was estimated based on % reduction in CD69 mean fluorescent intensity compared to stimulated control cells.

Results CD4+CD28null T cells (median % suppression: 40.1%) displayed lower sensitivity towards glucocorticoid-mediated suppression compared to CD28+ counterparts (median: 54.7%), seen in all individuals tested. Similarly, CD4+CD28null T cells (median: 17.5%) were less sensitive towards Tregs mediated suppression compared to CD28+ counterparts (median: 34.4%) in all individuals. No clear trend could be observed in CD8 compartment so far.

Conclusions Although, more individuals need to be tested, the above in vitro data support our in vivo findings that CD28null T cells are relatively resistant to glucocorticoid and Tregs mediated immunosuppression. Lower sensitivity of CD28null T cells towards glucocorticoid and Tregs mediated suppression support their treatment resistance nature in myositis and a role in chronic inflammation and autoimmunity.

A3.12 INTRARENAL Foxp3+ REGULATORY T CELLS EXPANSION AND DECREASED NUMBER OF INFILTRATING CD4+ T CELLS IN MURINE LUPUS BY IL-2 THERAPY

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Background and Objectives Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by an acquired IL-2 deficiency, which leads to a homeostatic imbalance between regulatory T cells (Treg) and effector T cells (Tcon). Humrich *et al*, (2010) demonstrated that the IL-2 deficiency in diseased (NZB × NZW) F1 mice can be rebalanced in lymphoid organs using a treatment with recombinant IL-2 (IL-2) by promoting the homeostatic proliferation of regulatory T cells. The aim of this study was to investigate the impact of IL-2 therapy on intrarenal Foxp3+ Treg and kidney infiltrating CD4+ cells in (NZB × NZW) F1 mouse model of lupus nephritis.

Materials and Methods (NZB × NZW) F1 mice with active nephritis were treated with recombinant IL-2 either over a short period or for a total of 30 days. Absolute numbers, phenotype and proliferation of kidney infiltrating CD4+ T cells were determined by flow cytometry.

Results (NZB × NZW) F1 mice treated over a short term with IL-2 showed an enhanced proliferation of Foxp3+ Treg and increased numbers and frequency of CD4+Foxp3+ Treg compared to untreated treated control mice. On the other hand, long term IL-2 treatment did not result in a persistent expansion of the intrarenal Foxp3+ Treg population. Nevertheless, total numbers of kidney infiltrating CD4+ T cells were diminished and the CD4+ T con showed reduced signs of cellular activation.

Conclusions Our data indicates that short term IL-2 treatment is able to expand the size of the intrarenal Treg pool. In contrast, long term IL-2 treatment decreases the numbers of kidney infiltrating CD4+ T cells. These results may in part explain the delay of disease progression induced by treatment with IL-2 and underline the important role of intrarenal Treg for the suppression of kidney disease in lupus mice. These results also provide additional rationales for an IL-2 based immunotherapy of human disease.