expanded in the PB of pSS, display an in vivo activated Th17 phenotype, infiltrate MSG and are resistant to corticosteroids. Taken together, these data suggest a key role of this T-cell subset in the perpetuation of chronic sialoadenitis and eventually in SS prognosis and provide the clue to target DN T cells for therapeutic purposes in pSS.

A3.5 COMBINATION BLOCKING OF IL-6 AND IL-21 IN EXPERIMENTAL ARTHRITIS INHIBITS THEIR REDUNDANT ROLE IN TH17-DRIVEN JOINT PATHOLOGY

doi:10.1136/annrheumdis-2013-203216.5

[1]Marije I Koenders, [1]Debbie M Roeleveld, [1,2]Renoud J Marijnissen, [3]Cheryl Nickerson-Nutter, [1]Fons van de Loo, [1]Wim B van den Berg. *[1]Radboud University Nijmegen Medical Centre, Rheumatology Research and Advanced Therapeutics, Nijmegen, the Netherlands; [2]University Medical Centre Utrecht, the Netherlands; [3]Pfizer Inc, Cambridge, Massachusetts, USA*

Background and Objectives Both IL-6 and IL-21 have been described to drive in vitro Th17 differentiation in the presence of TGFbeta. We explored whether also in vivo IL-6 and IL-21 play an exchangeable and redundant role in Th17 differentiation during experimental arthritis, and to what extent combined blocking of these cytokines inhibits Th17 differentiation and suppresses arthritis development.

Materials and Methods To investigate the synergistic effects of combined blocking of the IL-6 and IL-21 pathways, arthritis development and Th17 cells were first studied in IL-$6^{-/-}$xIL-$21R^{-/-}$ mice and their wild-type (WT) and single-knockout controls during antigen-induced arthritis (AIA). In addition, the kinetic effects of combined IL-6R and IL-21 neutralisation was studied in DBA-1J mice treated at various stages of collagen-induced arthritis (CIA).

Results Mice deficient for either IL-6 or IL-21R showed suppressed antigen-induced arthritis compared to WT controls. This disease reduction was accompanied by a significant reduction in CD4+IL17+ T cells in the draining lymph nodes as determined by FACS. However, mice lacking both the IL-6 and IL-21 signalling pathways showed an even stronger disease suppression than the IL-$6^{-/-}$ and IL-$21R^{-/-}$ mice, and a striking reduction in Th17 levels was observed in these mice.

Based upon our findings in gene-knockout mice, we aimed to confirm the synergistic effects of IL-6/IL-21 with a cytokine-neutralisation approach using anti-IL-6R antibodies and sIL-21R-Fc treatment during CIA. Antibodies were given as single treatment or in combination, and was started at immunisation (day 0) or around the booster (day 21).

Combined blocking of IL-6R and IL-21 early during arthritis development (day 0) was a very potent approach to prevent arthritis development, reaching a disease incidence of only 40% at day 35 (isotype control 100%, sIL-21R-Fc 100%, anti-IL-6R 60%). Analyzing the mice that did develop arthritis, we observed that the anti-IL-6R/sIL-21R-Fc combination was also clearly more potent in suppressing the arthritis severity in comparison to the single treatments. Interestingly, blocking the IL-6/IL-21 pathways at a later stage during arthritis development (day 21) was clearly less effective and did not show any additional effects to anti-IL-6R treatment alone.

Conclusions Combined blocking of the IL-6 and IL-21 pathways suppresses Th17 differentiation in vivo as demonstrated by our IL-6/IL-21R-deficient mice. However, our neutralisation study during CIA shows that to influence arthritis development this IL-6/IL-21 blocking approach only has a limited therapeutic window. These findings suggests that to target Th17-driven joint pathology, blocking Th17 effector cytokines like IL-17 and IL-22 might be more effective than attempting to reduce Th17 cell numbers during active disease.

A3.6 COMPARATIVE ANALYSIS OF THE THERAPEUTIC POTENTIAL OF INDUCIBLE TREG CELL POPULATIONS IN EXPERIMENTAL MODEL OF ARTHRITIS

doi:10.1136/annrheumdis-2013-203216.6

[1]Julie Quentin, [1]Delphine Martire, [1]Anne-Laure Mausset-Bonnefont, [2]Hélène Asnagli, [2]Nathalie Belmonte, [2]Arnaud Foussat, [1]Christian Jorgensen, [1]Rita Vincente, [1]Pascale Louis-Plence. *[1]INSERM U844, Université Montpellier 1, CHU Lapeyronnie, Montpellier, France; [2]TxCell, Allée de la Nertière, Les Cardoulines, 06 560 Valbonne-Sophia Antipolis, France*

Background and Objectives Adoptive cell transfer of Treg cells is a promising approach to restore tolerance in autoimmune disease. However the various type of Tregs, their doses of injection and their in vivo-suppressive mechanism need to be precisely define to clearly establish which Tregs will be able to dampen efficiently the immune response in the various settings.

In our study, we compared the therapeutic potential of IL10-secreting Tregs: Tr1 and CD49b-induced Tregs and $CD25^{+}FoxP^{+}$ Tregs. These two Treg populations share several phenotypic markers as well as immunosuppressive properties. In the present study we perform adoptive cell transfer experiments of the various inducible Treg cells in order to compare their impact on the immune response.

Materials and Methods CD49b Treg cells were generated in naive mice following repetitive injections of immature dendritic cells (DC). Cell sorting experiments were realised to obtain 98% pure CD49b T cells and $CD25^{+}$ cells. Collagen type II (bCII) specific Tr1 clones were obtained from TCR transgenic mice and expanded in vitro. Selected clones showed in vitro antigen specificity, Tr1 cytokine profile and IL10- and TGFβ-dependent suppressive activity. Several doses of CD49b or Tr1 cells were injected i.v. at day 28 in established collagen-induced arthritis. Clinical signs of arthritis were scored, as well as the in vivo impact on the proliferation of Teffector cells and the maturation of DC.

Results We defined for both Treg cell populations the most efficient dose in curative settings experiments. One single dose of 3×10^6 or 1×10^6 of Tr1 cell administration could reduce the incidence and severity of CIA. Interestingly, higher dose of 10M of Tr1 cells did not improve the disease. In the same manner, the dose of 10^5 $CD4CD49b^{+}$ cells reverse clinical symptom with a lack of efficacy of higher doses. We demonstrated following injection of Treg cells a clear impact on the proliferation of the effector cells in vivo.

Conclusions Our results suggest that even if the Treg cells present some similarities, we need to precisely define the dose and type of Treg that will be efficient in each experimental setting. We provide also a comparative analysis of the in vivo mechanism responsible of the protection of the various subtypes of Treg cells.

A3.7 COMPARISON OF THE EFFECTS OF TH17 AND TH1 CELLS ON ENDOTHELIAL CELLS AND SYNOVIOCYTES

doi:10.1136/annrheumdis-2013-203216.7

[1]Arnaud Hot, [2]Laura Maggi, [1]Fabien Lavocat, [2]Francesco Annunziato, [1]Pierre Miossec. *[1]Department of Clinical Immunology and Rheumatology; Immunogenomic and Inflammation Research Unit EA 4130, Hopital Edouard Herriot, University of Lyon, France; [2]Department of Internal Medicine and DENOTHE Centre, University of Florence, Firenze, Italy*

Background Interaction between different types of T-cells and surrounding non-hematopoietic cells is essential for the proper function of the immune system. Here, we focus on the synoviocytes from RA patients and Endothelial Cells (EC) and compare their interaction with Th1 and Th17 cells.

Methods To assess the interaction of T cells with stromal cells, the effects of Th1 or Th17 cytokines, Th1 or Th17 clone supernatants and coculture of Th clones and stromal cells were analysed. HUVEC (Human Umbilical Vein Endothelial Cells) were used as a model for EC and synoviocytes were isolated from synovium from RA