Methods: GLC mice were developed on a mixed genetic background (129Ola/Hsd-C57BL/6) by Cre-technology using LysM-promotor to knockout the *Pggt1b* gene in Mø[2]. CD4⁺ cells were isolated from spleen and lymph node (LN) of 16 weeks-old mice (GLC n=7, wt n=5) expected to have high prevalence of arthritis. RNA was extracted to measure expression of the Rho proteins and signature genes to characterize differences in Th-subtypes and migration abilities of CD4⁺ cells between GLC and wt mice. Furthermore, Illumina RNAseq analyzed the transcriptome of LN CD4⁺ cells. In a separate experiment we treated GLC mice with CTLA4-FP (n=12) or PBS (n=11) for 20 weeks from the age of 5 weeks. Rationale was to disrupt Mø/T cell contact to prevent arthritis. To study Rho-protein dependent phenotype in human RA, we performed RNAseq of sorted CD4⁺ cells of RA patients.

Results: RNAseq showed that CD4⁺ cells in LN of GLC mice had IFN- γ dependent cytotoxic profile and upregulated numerous pro-inflammatory genes including *Eomes*, *Cxcr3*, *Tigit*, *Tnfrsf10*, *Il-1r1*, *Stat1*, *Jak3*, *Irf7*, *Irf5*, *Ptpn13*. Furthermore, the over-represented genes often depended on the IRF family in their transcription.

GLC mice overexpressed *Cdc42* and *Rac1* in spleen CD4⁺ compared to wt (p=0.005 and p=0.048 resp.). Spleen GLC CD4⁺ cells had higher levels of $\alpha 5\beta 1$ and $\alpha 2\beta 2$ integrins, strongly correlating to *Cdc42* (r=0.61 p=0.0027 and r=0.50, p=0.018) and arthritis (r=0.64, p=0.0015 and r=0.69, p=0.0004). Importantly, *Cdc42*, *Rac1*, and *RhoA* were higher expressed in LN CD4⁺ compared to spleen (p=0.016, p=0.031 and p=0.016). In addition, *Itgb1* coding for $\beta 1$ integrin, was upregulated in GLC CD4⁺ cells of both spleen and LN (p=0.003 p=0.03, resp.), suggesting Rho proteins are important for migration of CD4⁺ cells to the joint draining LN and for arthritis development. CD4⁺ cells that migrated to the LN had high proportion of Foxp3⁺ cells. This also correlated to the expression of *Itgb1* (r=0.84, p=0.0012) presenting a plausible mechanism for increased influx of Tregs into joints. Several observations are in favor of this notion. First, GLC mice expressed more *Foxp3* in LN compared to spleen CD4⁺ cells (p=0.016). Second, transcription of *Foxp3* in LN CD4⁺ cells was higher in GLC mice compared to wt (p=0.015). Third, this high *Foxp3* coexisted with low transcription of *Lef1* (p=0.03), required for Treg immunosuppression. Last, *Foxp3* correlated negatively to both *Lef1* (r=-0.72, p=0.017), and its cofactor *Tcf1* (r=-0.75, p=0.01).

CTLA4-FP reduced inflammation in GLC mice evident as lower IFN- γ , IL-6 and TNF- α production (p=0.0002, p<0.0001 and p<0.0001 resp.) and the number of CD25⁺CD4⁺ cells in spleen (p=0.027). In contrast, we observed increased IL-17A production (p=0.056). However, CTLA4-FP treatment did not affect migration of CD4⁺ cells enriched with Rho-protein into draining LN nor alleviate arthritis.

Similar to the GLC mice, CD4⁺ cells of RA patients with high expression of RhoA, Rac1 and Cdc42 demonstrated enrichment for Th1 signature genes including *IFNG*, *TBX21*, *Eomes*, *IL2RA*, *IL2RB*, *IL12RB2*, *TNF*, *IL18RAP* (all, adj. p<0.05).

Conclusion: This study shows that accumulation of Rho-proteins in CD4⁺ cells results in pro-inflammatory IFN- γ dependent phenotype in mice and human RA. Accumulation of RhoA, Rac1 and Cdc42 proteins trigger the migration of CD4⁺ cells into joint draining LN and facilitates arthritis. Inhibiting Mø/T cell contact in GLC mice did not suffice to prevent migration of Rho-protein expressing cells and arthritis

REFERENCES:

- [1] Khan, O.M., et al. J Clin Invest. 2011. 121(2): p. 628-39.
- [2] Akula, M.K., et al. Nat Commun. 2019. 10(1): p. 3975.

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OP0023

GENERATION OF PT101, A HIGHLY SELECTIVE IL-2 MUTEIN FOR TREATMENT OF AUTOIMMUNE DISEASES

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Background: Regulatory T cells (Tregs) play a critical role in immune homeostasis and are dysfunctional in many autoimmune diseases. Interleukin 2 (IL-2) drives the proliferation and function of Tregs. via the heterotrimeric IL-2 receptor (CD25/CD122/CD132). As a result, CD25 loss-of-function in mice is associated with Treg deficiency and widespread autoimmunity. Low dose IL-2 is being evaluated for treatment of autoimmune diseases and has been shown to expand Tregs, however it has a narrow selectivity window before activating conventional T cells and natural killer cells. To enhance IL-2 selectivity and improve its therapeutic utility for activating and expanding Tregs, mutations can be introduced that reduce CD122/CD132 affinity thus creating a dependency on CD25 binding

for signaling through CD122/CD132 upon IL-2 facilitated CD25/CD122/CD132 trimer formation.

Objectives: To generate a highly selective IL-2 mutein that activates and expands Tregs selectively that can be used for treatment of autoimmune diseases

Methods: Using a structure guided approach, we introduced mutations in IL-2 that significantly decreased CD122 binding affinity in addition to other mutations that increased CD25 binding affinity. Finally, we explored additional mutations, format, orientation, and linker lengths to generate the most potent, selective molecule with drug-like manufacturability. These structure activity relationship efforts culminated in the generation of PT101, a mutant IL-2 Fc fusion that is selective in activating and expanding Tregs in vitro and in vivo.

Results: PT101 selectively induced STAT5 phosphorylation in human and cynomolgus monkey Tregs in vitro. In humanized NOD-scid IL-2R γ -null (NSG) mice and cynomolgus monkeys, administration of PT101 dose-dependently and selectively expanded Tregs without significant effects on other immune cell types, and without eliciting proinflammatory cytokine production. The Tregs from PT101-dosed humanized mice have increased expression of FOXP3 and CD25, suggesting enhanced function and stability. In a Phase 1a single ascending dose clinical trial, PT101 was well-tolerated and selectively expanded total Tregs, with a mean maximal increase of up to 3.6-fold over baseline in healthy volunteers. There was no evidence of expansion of natural killer cells nor pro-inflammatory conventional T cells at any of the doses studied.

Conclusion: PT101 selectively activated and expanded Tregs without significant effects on other immune cell types, and without eliciting proinflammatory cytokine production. These Tregs have enhanced function and stability as seen by increase in expression of FOXP3 and CD25 in these cells. PT101 maintained selectivity in Phase 1 a clinical trial with no evidence of expansion of natural killer cells nor pro-inflammatory conventional T at any dose studied. A Phase 1b/2a clinical trial in patients with ulcerative colitis and a Phase 2 clinical trial in patients with systemic lupus erythematosus are planned to further evaluate PT101.

Disclosure of Interests: None declared

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OP0024

DECREASED LEVELS OF T FOLLICULAR HELPER (CD4+CXCR5+) CELLS AND CD27+CD38+ AND CD27+CD38- B CELLS IN ANKYLOSING SPONDYLITIS PATIENTS CORRELATE WITH MARKER OF INFLAMMATION

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Background: The role of different lymphocyte subsets in ankylosing spondylitis (AS) is still to be elucidated. It has previously been reported contradictory data concerning the levels of T Follicular Helper (TFH) cells and differentiated B cells in peripheral blood of AS patients. In addition, the connection to disease related parameters is still to be fully revealed.

Objectives: The purpose of this study was to investigate the level of CD4+TFH cells and CD27+CD38+/CD38- B cells in patients with AS from northern Sweden and to compare the levels with age and sex-matched controls. We also studied associations between these cell subsets and disease related factors.

Methods: Peripheral blood mononuclear cells (PBMCs) from a cohort of 50 patients with AS from Region Västerbotten (mean age 52±9.1 years, 33 (66 %) men, 50 (100 %) HLAB27 positive) and 50 pair wise matched blood donor controls (mean age 54±8.8 years, 33 (66 %) men) were stained with a combination of antibodies allowing for the detection of CD27, CD38, CD19, CD3, CD4 and CXCR5 markers and analyzed by flow cytometry. In addition, the patient with AS were examined with spinal x-ray for radiographic alterations assessed with mSASSS. CRP and ESR were measured and physical function and disease activity were registered with BASMI and BASFI respectively ASDAS-CRP and BASDAI.

Results: When comparing AS patients and controls pair wise, we observed on average a 50% reduction of TFH (CD3+CD4+CXCR5+) cells among CD45+ lymphocytes in PBMCs from patients (p=0,000008). Furthermore, a 20-30% reduction among memory/plasma cells (CD19+CD27+CD38+ and CD19+CD27+CD38-) among CD45+ lymphocytes in PBMCs from patients (p=0,002 and p=0,007 respectively). For female patients a correlation between TFH and ESR (R_s = 0,551 p=0,022) was observed. Moreover, negative correlations between the two B cell subsets (CD19+CD27+CD38+ and CD19+CD27+CD38-) and ESR were observed for female patients (R_s = -0,476 p=0,053 and R_s = -0,522 p=0,032 respectively).

Conclusion: TFH cells was reduced in AS patients and this reduction correlated with a reduction in differentiated (CD27+CD38+ and CD27+CD38-) B cells. In