Confirmation several targets of HOXD10, -D11, and -D13 by qPCR, e.g. NR4A1, ROR2, LF/ATF3.

Figure 1. Comparison of the genes which were differentially expressed after HOXD10-11-13 silencing

Conclusion: The expression of HOXD10, -D11, and -D13 in synovial fibroblasts and tissues strikingly overlaps with predilection sites for RA. Silencing experiments suggested that these embryonic HOX transcription factors have a crucial role in regulating fibroblast functions and might shape a joint specific environment that modulates the development and course of RA in specific joints.

REFERENCES:

Disclosure of Interests: Masoumehalsadat Mirahimi: None declared, Kerstin Klein: None declared, Miranda Houtman: None declared, Malgorzata Maciukiewicz: None declared, Mojca Frank Bertoncelj: None declared, Astrid Juengel: None declared, Martin Berli: None declared, Miriam Marks: None declared, Olivier Distler Speakers bureau: 1-Speaker fee on Scleroderma and related complications: Bayer, Boehringer Ingelheim, Medscape, Novartis, Roche • Speaker fee on rheumatology topic other than Scleroderma: MSD, iQone, Novartis, Pfizer, Roche, Consultant of: • Consultancy fee for Scleroderma and its complications: Abbvie, Acceleron Pharma, Amgen, AnMar, Anx Therapeutics, Bayer, Baeccon Discovery, Boehringer, CSL Behring, ChemomAb, Corbus Pharmaceuticals, Horizon Pharmaceuticals, Galapagos NV, GSK, Glenmark Pharmaceuticals, Inventiva, Italfarmaco, IQvia, Kymera, Medac, Medscape, Mitsubishi Tanabe Pharma, MSD, Roche, Roivant Sciences, Sanofi, UCB • Consultancy fee for rheumatology topic other than Scleroderma: Abbvie, Amgen, Lilly, Pfizer, Grant/research support from: • OD has/had consultancy relationship and/or has received research funding in the area of potential treatments for systemic sclerosis and its complications from (last three years): Abbvie, Acceleron Pharma, Amgen, AnMar, Anx Therapeutics, Baeccon Discovery, Blade Therapeutics, Bayer, Boehringer Ingelheim, ChemomAb, Corbus Pharmaceuticals, CSL Behring, Galapagos NV, Glenmark Pharmaceuticals, GSK, Horizon (Curzon) Pharmaceuticals, Inventiva, IQvia, Italfarmaco, IQone, Kymera Therapeutics, Lilly, Medac, Medscape, Mitsubishi Tanabe Pharma, MSD, Novartis, Pfizer, Roche, Sanofi, Serodapharm, Topadur, Target Bioscience and UCB. Patent issued “miR-28 for the treatment of systemic sclerosis” (US20124389, EP20331143).

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OP0021

IDENTIFICATION OF Differentially EXpressed GENES IN EARLY RheuMatoId ARTHRITIS PATIENTS RESPONDING TO TOCILIZUMAB


Methods: Two cohorts of RA patients were included: one cohort (n=14) and non-responders (n=7). For external replication, in a second cohort (n=95 therapy-naive patients receiving TCZ monotherapy), RNA-seq was conducted on baseline PBMC RNA (SMARTer Stranded Total RNA-Seq Kit, Takara Bio) from the 2-year, multicenter, double-blind, placebo-controlled, randomized U-Act-Early trial (ClinicalTrials.gov identifier: NCT01344737) and DGE was analyzed between 84 responders and 11 non-responders.

Results: Whole blood DGE analysis showed two significantly higher expressed genes in TCZ non-responders (False Discovery Rate, FDR < 0.05): 10.1136/annrheumdis-2021-eular.1323

Disclosure of Interests: None declared

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Adaptive immunity (T cells and B cells) in rheumatic diseases and In innate immunity in rheumatic diseases

OP0022

RHO EXPRESSION FACILITATES T CELL MIGRATION TO LYMPH NODES IN RESPONSE TO INFLAMMATION

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Background: Deficiency in geranylgeranylationtransferase type I (GGTase-I) results in accumulation of active Rho family proteins RhoA, Rac1 and Cdc42, responsible for cell communication and migration. We reported that mice with GGTase-I deficient macrophages (GLC mice) develop a spontaneous and age-dependent arthritis, reproducing pathology of RA [1].

Objectives: We study how GGTase-I deficiency in Ma changes T cell phenotype to facilitate their translocation to joints and the development of arthritis.

Disclosure of Interests: None declared

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Methods: GLC mice were developed on a mixed genetic background (129Ola/Hsd-C57BL/6) by Cre-technology using LysM-promotor to knockout the Pptg1b gene in Mx2. CD4+ cells were isolated from spleen and lymph node (LN) of 16 weeks old mice (GLC n=7, wt n=6) 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-FF (n=12) or PBS (n=11) for 20 weeks from the age of 5 weeks. Rationale was to disrupt Mo/Te 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 cytokotic profile and upregulated numerous pro-inflammatory genes including Eomes, Cxcl3, Tgif, Tnfalfa, Ifn-1, Ifi3, Jnk3, Ifit5, Pitpnr13. 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 α5β1 and α2β1 integrins, strongly correlating to Cdc42 (r=0.06; 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, Tgft1 coding for j1 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 Fopx3+ cells. This also correlated to the expression of Tgft1 (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 Left1 (p=0.03), required for Treg immunosuppression. Last, Foxp3 correlated negatively to both Left1 (r=-0.72, p=0.017), and its cofactor Tcf7 (r=-0.75, p=0.01).

CTLA4-FF reduced inflammation in GLC mice evident as lower IFN-γ, IL-6 and TNF-α production (p=0.0002, p=0.0001 and p=0.001 resp.) and the number of CD25+CD69+ cells in spleen (p=0.027). In contrast, we observed increased IL-17A production (p=0.056). However, CTLA4-FF treatment did not affect migration of CD4+ cells enriched with Rho-protein into draining LN nor alleviated 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, IL2RBB, 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 from joint draining LN and facilitates arthritis. Inhibuting Mo/Te cell contact in GLC mice did not suffice to prevent migration of Rho-protein expressing cells and arthritis.

REFERENCES:

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DECREASED LEVELS OF T FOLLICULAR HELPER (CD4+CXCR5+) CELLS AND CD27+CD38+ AND CD27-CD38- B CELLS IN ANKYLOSING SPONDILITIS 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+ 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±1.1 years, 33 (66 %) men, 50 (100 %) HLA-B27 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, CD4 and CD122. Flow cytometry analysis was performed, and data was compiled using FlowJo. Associations between these cell subsets and disease related factors were evaluated using Pearson correlation analysis.

Results: The study included 50 patients with AS and 50 age and sex matched controls. The percentage of TFH cells among CD4+ T cells was increased in AS compared to controls (p=0.03). CD38+ B cells were significantly decreased when comparing AS patients and controls pair wise (p=0.01). There was a significant negative correlation between age and CD27+CD38- B cells (r=-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=-0.476 p=0.053 and r=-0.522 p=0.032 respectively).

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