Methods: GLC mice were developed on a mixed genetic background (129Ola/Hsd-C57BL/6) by Cre-technology using LysM-promoter to knockout the Pgygt1b gene in Mx2. CD4+ cells were isolated from spleen and lymph node (LN) of 16 weeks old mice (8–10/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, Cxcl3, Tgf, Tnfa, Ifn-γ, Stat1, Jak3, Itf, 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 αS1(1-2) integrins, strongly correlating to Cdc42 (r=0.41 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, Itgβ7 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 Foxp3+ cells. This also correlated to the expression of Itgβ7 (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 Lefr1 (p=0.03), required for Treg immunosuppression. Last, Foxp3 correlated negatively to both Lefr1 (r=-0.72, p=0.0017), and its co-factor Tcf7 (r=-0.75, p=0.01).

CTLA4-FP reduced inflammation in GLC mice evident as lower IFN-γ, IL6 and TNFα production (p=0.0002, p=0.0001 and p=0.001 resp) and the number of CD25+CD4+ cells in spleen (p=0.072). 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 to joint draining LN and facilitates arthritis. Inhibiting Mø cell contact in GLC mice did not suffice to prevent migration of Rho-protein expressing cells and arthritis.

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addition, the inflammation marker ESR was negatively correlated with TFH as well as with the differentiated CD14 cells in female patients. Our observations indicate a role of the humoral immune response in AS.

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Background: Dendritic cells (DCs) are a heterogeneous population of professional antigen-presenting cells which are at the interface between innate and adaptive immunity. A specific subset of DCs is known to derive from monocyte differentiation and have a key role in inflammation and infection. Objectives: This study aimed to characterize the phenotype and function of a distinct CD209+/CD14+ DC subset in the periphery and at the site of inflammation in patients with rheumatoid arthritis (RA) and psoriatic arthritis (PsA), in addition to examining the effect Tofacitinib and TNF inhibitor on their development.

Methods: Peripheral blood and synovial fluid mononuclear cells (PBMC and SFMC) were isolated by Ficoll density gradient from healthy subject (HC), and patients with RA and PsA. Single cell synovial tissue suspension (ST) from RA and PsA patients were also established using enzymatic/mechanical digestion. PBMC, SFMC and ST were analysed by flow cytometry to identify the CD209+/CD14+ DC subset, its frequency and the cell surface expression of chemokine receptors (CCR6, CCR7, CXCR3, CXCR4 and CXCR5) and activation markers (CD40 and CD80) in RA/PsA. Furthermore synovial tissue single cell analysis from RA/PsA patients was performed by single cell synovial tissue analysis (SCSFA) using the Cell-OF-ONE platform. In addition, we performed CD3, CD19 and CD56 depletion of RA/PsA PBMC followed by SPICE analysis further identified differential expression and co-expression of CXCR3 and CXCR5 compared to the HC. Furthermore synovial tissue single cell analysis from RA/PsA patients was performed by single cell synovial tissue analysis (SCSFA) using the Cell-OF-ONE platform. In addition, we performed CD3, CD19 and CD56 depletion of RA/PsA PBMC followed by SPICE analysis further identified differential expression and co-expression of CXCR3 and CXCR5 compared to the HC. Interestingly, this distinct DC population was significantly enriched at the site of inflammation, in both SFMC and ST, displaying a more mature phenotype, evident by the observed significant increase in CD40 and CD80 expression. SPICE analysis further identified differential expression and co-expression of chemokine receptors at the periphery of RA and PsA patients, when compared to the HC. Furthermore synovial tissue single cell analysis from RA/PsA demonstrated a unique chemokine receptors profile demonstrating increased single expression and co-expression of CXCR3 and CXCR5 compared to the periphery. Finally, we have previously observed that JAK/STAT is involved in monocyte-derived dendritic cell population development (1,2), therefore we performed CD3, CD19 and CD56 depletion of RA/PsA PBMC followed by stimulation with GMCSF/IL4 to stimulate the Mo-DC population, in the presence of Tofacitinib or Humira. Interestingly, we observed that JAK/STAT inhibition, but not TNF inhibitor, reduced the generation and development of CD209+/CD14+ DC.

Conclusion: We identified, for the first time, a distinct CD209+/CD14+ DC population in PBMC of patients with RA and PsA, with similar frequency across the groups. However, when PBMC were stimulated with TLRs, an increase of IL12 and TNF− was observed in RA and PsA PBMC when compared to the HC. Interestingly, this distinct DC population was significantly enriched at the site of inflammation, in both SFMC and ST, displaying a more mature phenotype, evident by the observed significant increase in CD40 and CD80 expression. SPICE analysis further identified differential expression and co-expression of chemokine receptors at the periphery of RA and PsA patients, when compared to the HC. Furthermore synovial tissue single cell analysis from RA/PsA demonstrated a unique chemokine receptors profile demonstrating increased single expression and co-expression of CXCR3 and CXCR5 compared to the periphery. Finally, we have previously observed that JAK/STAT is involved in monocyte-derived dendritic cell population development (1,2), therefore we performed CD3, CD19 and CD56 depletion of RA/PsA PBMC followed by stimulation with GMCSF/IL4 to stimulate the Mo-DC population, in the presence of Tofacitinib or Humira. Interestingly, we observed that JAK/STAT inhibition, but not TNF inhibitor, reduced the generation and development of CD209+/CD14+ DC.

Conclusion: We identified for the first time a distinct monocyte-derived DC population characterized as CD209+/CD14+ in the periphery of RA and PsA patients. This population was enriched at the site of inflammation and displayed a unique chemokine receptor profile, suggesting that these cells are already activated in the periphery of IA patients, and are recruited and further activated in the inflamed joint. In addition, we showed that the CD209+/CD14+ DC development is regulated by JAK/STAT signalling, but not TNF inhibition.

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OP0026

IGF1R DEPENDENT CELL INTERACTION AND REGULATION OF AUTOANTIBODY PRODUCTION IN RHEUMATOID ARTHRITIS

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Background: The insulin-like growth factor 1 receptor (IGF1R) signalling mediates numerous developmental processes acting through downstream adaptor molecules IRS1/2, which activate Akt and inhibit the family of forhead box class O (FoxO). IGF1R signalling alliates rheumatoid arthritis (RA) (Erlandsson et al., 2017), however, the role of IGF1R signalling in the regulation of immune function is poorly understood.

Objectives: To investigate the link between IGF1R signalling and antigen presentation in experimental arthritis.

Methods: Arthritis was induced by immunising Balb/c mice with methylated bovine serum albumin (mBSA, n=18) and DBA/1 mice with type II collagen (CII, n=18). The mice were treated with a synthetic IGF1R inhibitor NT157 or with vehicle/ non-targeting (nt)RNA, respectively. Controls were treated with cycloheximide/ non-targeting (nt)RNA, respectively. Flow cytometry was used to isolate spleen cell phenotype. Antibody levels were measured by ELISA. Immunohistochemistry (IHC) of spleen was performed for assessment of marginal zone (MZ) and location of pS6/IRS1+ and pS6/FOXO1+ cells. IHC images were acquired by fluorescent confocal microscopy, and analysed using ZEN2009 and Cell Profiler soft ware.

Results: The inhibition of IGF1R resulted in an 80% increase in MZ area in NT157-treated mice compared to controls (p=0.0001). This was supported by a significant increase of CD21+ (p=0.034) and CD23+ cell populations (p=0.00059), both among the CD19+ B cells and antigen-presenting MCHI+CD19- cells, implying that IGF1R expression regulates the populations of MZ and follicular cells. Additionally, there was a strong positive correlation between the decrease of IGF1R+ and ICOSL+ population on CD21+ cells (r=0.70, p=0.0071), which retained them in the MZ and prolonged communication with macrophages. Insufficient feedback from ICOSL- B cells limited expression of CXCR5 on CD4+ cells. The IHC analysis displayed that, IGF1R inhibition led to abundance of inactivate pS612IRS1 molecules IRS1/2, which activate Akt and inhibit the family of forkhead box class O (FoxO). Inhibition of IGF1R signalling alleviates rheumatoid arthritis (RA) (Erlandsson et al., 2017), however, the role of IGF1R signalling in the regulation of immune function is poorly understood.

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OP0027

AS-RELATED TCR Beta CLONOTYPES ARE PRESENT IN DISCONTINUOUSLY INFILTRATED TISSUES OF PATIENTS WITH SPONDYLOARTHROPATHIES

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