tenascin specific CD4+T cells, followed by PHA expansion resulted in visible increase in proportion of citrullinated tenascin specific CD4+T cells.

Disclosure of Interests: Ravi kumar: None declared, Niyaz Yoosuf: None declared, Christina Gerstner: None declared, Sara Turcinov: None declared, Karine Chemin: None declared, Vivianne Malmström Grant/research support from: VM has had research grants from Janssen Pharmaceutica
DOI: 10.1136/annrheumdis-2020-eular.1093

THU0047

THE SYNOVIUM REQUIRES AN IMMUNOLOGICAL RHEOSTAT THAT DEFINES TWO FUNCTIONALLY DISPARATE PATHOGENIC CD4+HLA-DR+ SUBSETS IN HUMAN ARTHRITIS

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Background: Despite advances in understanding how the adaptive T cell landscape is affected in human arthritis, specific T cell subset knowledge has yet to be utilised in clinical settings. We have previously discovered within active arthritic patients, a circulating pathogenic-like lymphocyte (CPLs; CD4+HLA-DR+) within the T-effector compartment, that is phenotypically similar to their synovial counterparts. CPLs are inflammatory, correlate with disease activity and overlap in synovial TCR repertoire. A similar inflammation-associated T-regulatory (iaTreg; CD4+HLA-DR+) subset, that is activated, poised to migrate to inflamed site and sharing synovial TCR overlap, suggest a common disease ontogeny that may exist between CPLs and iaTregs.

Objectives: Here we seek to determine whether and how the synovial microenvironment plays a role in modulating these two functionally divergent (Teff/Treg compartments) yet pathogenically homologous subsets. This modulation, akin to an immunological rheostat, may be a feature of the disease process.

Methods: We examined CD45+ immune cells from synovial and PBMCs (active JIA, inactive JIA, paediatric healthy) through mass cytometry (CyToF). CD4 T cells were sorted into CPLs, iaTregs, Teff and Treg through FACS Aria II, from active JIA PBMCs, paired JIA SFMCs and healthy paediatric PBMCs and examined through ngRNAseq.

Results: Mass cytometric analysis reveal a significant enrichment of synovium signatures in both circulatory CPLs and iaTregs subsets from active arthritic PBMCs, as compared with the conventional pool of Teff/Tregs. This immunological relationship between CPLs/iaTregs is reaffirmed by comparative differential gene expression (DEG) and phylogenetic tree analysis, which indicated transcriptomic convergence between circulatory pathogenic CPLs/iaTreg subsets and divergence from their respective conventional Teff/Treg pools. Circulatory CPLs/iaTregs exhibit (a) common pathway dysregulation in T cell signalling, (b) restriction in TCR oligoclonality and (c) common transcription factor drivers within the gene regulatory network, suggesting a common pathogenic mechanism acting on these two disparate compartments.

To understand how the microenvironment plays a role in modulating these two subsets, we compared the transcriptome of CPLs/iaTreg and conventional Teff/Treg subsets from (a) healthy PBMCs, (b) JIA PBMCs and (c) paired JIA SFMCs. The convergence between CPLs/iaTreg increases across the spatial/disease continuum, culminating in 7 key common dysregulated pathways within synovium CPLs/iaTregs. Importantly we detected higher clonotypic sharing of TCRs in CPLs/iaTregs across the spatial and disease continuum, suggesting a common precursor driven by antigenic selection.

Conclusion: Our data suggest that CPLs/iaTregs are dichotomic components of a systemic immune rheostat, shape through the synovium environment, modulating autoimmunity in human arthritis. As iaTreg and CPL most likely have the capacity to morph into each other, the molecular crossroads which control this plasticity represent novel therapeutic targets.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.4006

THU0048

THE FUNCTION CHANGES OF SNPS IN THE P2X7 RECEPTOR BY ALA348TO THR, GLU496TO ALA AND ARG307TO GLN IN THP-1 CELLS WITH HIGH URIC ACID

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Background: Previous studies[1] have shown that ATP acts on the sputum receptor P2X ligand-gated ion channel 7 (P2X7R) as a second signal to induce gouty arthritis.

Objectives: In this study, the functional changes of three SNP genotypes, Ala348 to Thr, Glu 496 to Ala and Arg307 to Gln, in P2X7R were analyzed with high uric acid.

Methods: After transfection of HEK-293T cells by lentivirus, observing the uptake ability of HEK-293T cells to ethidium bromide. The effect of three different mutants on the P2X7 receptor was thus observed on the P2X7 channel. In addition, THP-1 cells were also transfected, stable expression of a THP-1 cell line that has been transfected with a wild-type or different mutants and thus established. Then three types were set up separately, and each type was randomized into three groups: MSU(labeled M), MSU+ATP (labeled MA), and unstimulated control group (labeled C). Detection of IL-1 β protein expression level in serum by ELISA and NLRP3, ASC and Caspase-1 mRNA levels in transfected THP-1 cells by qRT-PCR.

Results:

1. These three variants have different effects on the uptake function of ATP-induced ethidium⁺ bromide in transfection of HEK-293T cells by lentivirus. Ala³⁴⁸to Thr increased P2X₇-dependent ethidium⁺ bromide uptake (145% of wild-type P2X₇ response, P<0.001). In contrast, Absent or very reduced P2X₇ function was found in Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln subjects, appeared to abolish P2X₇-dependent dye uptake (38% and 32% of wild-type P2X₇ responses, P<0.001.), who were compared with wild-type.
2. Compared the IL-1 β levels of the three variants with the wide-type and empty virus in THP-1 cells, the Ala³⁴⁸to Thr mutation significantly up-regulated the serum levels of IL-1 β compared with the wide-type and empty virus in group MA with high uric acid (P=0.0007;P=0.013, respectively). Moreover, similar results have also been shown in IL-1 β mRNA expressions (P=0.0334; P=0.0307, respectively). The Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln mutations down-regulated the serum levels of IL-1 β compared with the wide-type in group MA (P=0.0189;P=0.0164, respectively).
3. NLRP3 mRNA was significantly increased in the Ala³⁴⁸to Thr mutation compared with the wide-type and empty virus in group MA (p =0.0003;P=0.0001, respectively). However, NLRP3 mRNA was significantly reduced in the Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln mutations compared with the wide-type in group MA (p =0.0294;P=0.0279, respectively).
4. Wild-type was significantly higher than empty virus in the ASC gene expression in group MA(P=0.0022). Moreover, the Ala³⁴⁸to Thr mutation was higher than empty virus while Arg³⁰⁷to Gln mutation was lower than that in group MA (P=0.0138;P=0.0283, respectively).
5. Unlike NLRP3 gene expression, the data showed that the expression of caspase-1 mRNA in group C, M and MA all with no statistical significance, respectively (P>0.05).

Conclusion: Our data revealed that Ala³⁴⁸to Thr up-regulate the functional status of P2X7R and Glu⁴⁹⁶to Ala and Gln⁴⁶⁰to Arg down-regulate the functional status of P2X7R, which resulted in a significant increase or decrease in IL-1 β and NLRP3 expression levels with high uric acid.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1463

THU0049

DISTINCT EXPRESSION OF COINHIBITORY MOLECULES ON ALVEOLAR T CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS- AND IDIOPATHIC INFLAMMATORY MYOPATHIES-ASSOCIATED INTERSTITIAL LUNG DISEASE

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Background: Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA) and also the most common non-musculoskeletal manifestation of idiopathic inflammatory myopathies (IIM), including polymyositis, dermatomyositis and clinically amyopathic dermatomyositis.

Previous studies have suggested that alveolar macrophages (AMs) and T cells are associated with the pathogenesis of ILD. Recently, it is reported that coinhibitory molecules are expressed at the site of inflammation such as RA synovium; however, detailed lung immunophenotyping has not been reported.

Objectives: To identify immunologic factors in the lungs of patients with RA-associated ILD (RA-ILD) and IIM-associated ILD (IIM-ILD) and to examine their pathological mechanisms.

Methods: A total of 11 patients with RA-ILD, 16 with IIM-ILD, and 6 with drug-induced ILD (DI-ILD) and 8 healthy controls were enrolled. Peripheral blood and bronchoalveolar lavage fluid (BALF) were immunophenotyped by flow cytometry. AMs were analyzed by RNA-sequence and coculture assay with peripheral naïve CD4+ T cells of healthy individuals.

Results: Several coinhibitory molecules were coexpressed on BALF T cells in the order of CTLA-4, PD-1, Tim-3, and LAG-3 from most to least, whereas only PD-1 was expressed on peripheral T cells among them. In RA-ILD, PD-1+ and Tim-3+ CD4+ T cells in the BALF were increased. PD-1+CD4+ T cells populations correlated differentiated B cells and Tim-3+CD4+ T cells populations correlated with ILD severity and RF titer. In contrast, in IIM-ILD, activated CD8+ T cells were increased and they coexpressed CTLA-4, PD-1 and Tim-3. BALF PD-1+CD4+ T cells rarely expressed CXCR5, and they positively correlated with plasmablasts and plasma cells, indicating most of them are considered Tph cells. In the coculture experiments, AMs of RA-ILD and IIM-ILD induced more PD-1 and Tim-3 on CD4+ T cells, suggesting that coinhibitory molecule expression on BALF T cells was partly due to AMs. In RNA-sequence, PD-ligand (PD-L) 1 and PD-L2 genes were significantly downregulated in AMs from RA-ILD compared with DI-ILD.

Conclusion: We identified T cell subsets that play a central role in the pathogenesis of RA-ILD and IIM-ILD; PD-1 on T cells in RA-ILD and Tim-3 on CD8+ T cells in IIM-ILD might be key factors in the disease process. The evaluation of coinhibitory molecules on BALF T cells could be clinically useful.

Disclosure of Interests: Maho Nakazawa: None declared, Katsuya Suzuki: None declared, Masaru Takeshita: None declared, Jun Inamo: None declared, Hirofumi Kamata: None declared, Makoto Ishii: None declared, Yoshitaka Oyama: None declared, Hisaji Oshima: None declared, Tsutomu Takeuchi Grant/research support from: Eisai Co., Ltd, Astellas Pharma Inc., AbbVie GK, Asahi Kasei Pharma Corporation, Nippon Kayaku Co., Ltd, Takeda Pharmaceutical Company Ltd, UCB Pharma, Shionogi & Co., Ltd., Mitsubishi-Tanabe Pharma Corp., Daiichi Sankyo Co., Ltd., Chugai Pharmaceutical Co. Ltd., Consultant of: Chugai Pharmaceutical Co Ltd, Astellas Pharma Inc., Eli Lilly Japan KK, Speakers bureau: AbbVie GK, Eisai Co., Ltd, Mitsubishi-Tanabe Pharma Corporation, Chugai Pharmaceutical Co Ltd, Bristol-Myers Squibb Company, AYUMI Pharmaceutical Corp., Eisai Co., Ltd, Daiichi Sankyo Co., Ltd., Gilead Sciences, Inc., Novartis Pharma K.K., Pfizer Japan Inc., Sanofi K.K., Dainippon Sumitomo Co., Ltd.

DOI: 10.1136/annrheumdis-2020-eular.4542

THU0050 CXCL13 IS A KEY DRIVER FOR MIGRATION AND DIFFERENTIATION OF REGULATORY B CELLS

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Background: Regulatory B cells in human still need to be characterized. Given the absence of a phenotypical definition of these cells, a functional definition based on their ability to secrete IL-10 is often used (corresponding to B10+ cells). Chemokine receptors (CKR) profiles are useful to characterize some populations of T cells but have never been explored among B10+ cells. Moreover, very little is known about B10+ cell migration. Chemokines (CK) have also been implicated in the differentiation of naïve T cells towards regulatory T cells.

Objectives: Therefore, the aims of our study were to first characterize the profile of CKR on B10+ cells, and second to investigate CK implicated in their migration and differentiation, this, both in control (CTL) and in patients with rheumatoid arthritis (RA).

Methods: B cells were isolated with Rosette Sep Human B cells enrichment followed by Ficoll separation. B cells were then activated 24 hours with CpG and CD40L to generate B10+ cells. IL-10 secretion from B cells was assessed by FACs and ELISA. We compared the expression of several CKR between B10+ and IL-10^{neg} B cells (B10^{neg}) from CTL and RA patients by flow cytometry. For migration assay, B10+ and B10^{neg} cells were sorted by FACSaria. Their ability to migrate, in response to ligand of CKR found differentially expressed in the first part (CCL21, CCL22, CXCL11, CXCL12 or CXCL13) or synovial fluid (SF) from RA patients, were evaluated by migration assay in 5µM Transwell

chambers and expressed as fold increase compare to basal migration towards control media.

Results: B10+ cells expressed a different profile of CKR compared to B10^{neg} both in CTL and RA patients and these profiles differed between B10+ cells of CTL and RA patients. However, no CKR profile could phenotypically define B10+ cells. Of note, CXCR5 was under-expressed on B10+ cell surface compared to B10^{neg} in CTL (75% [IQR 72.9-81.4] positive cells among B10+ vs 99.2% [98.4-99.4] positive cells among B10^{neg}, p=0.006, n=10) and also in RA patients (78.3% [70.8-82.3] vs 98.2% [96.9-99.54], p=0.008, n=8). Nevertheless, mRNA expression of CXCR5 was higher among B10+ versus B10^{neg} cells in CTL and RA patients. As CpG-stimulated cells over-expressed CXCL13, ligand of CXCR5, we hypothesized that the binding of its ligand induced the internalisation of CXCR5. Indeed, among all CK tested, only CXCL13, attracted significantly more B10+ than B10^{neg} from CTL (9.1 [5.6-14.6] fold increase migration of B10+ vs 5.2 [3.1-7.5] fold increase migration of B10^{neg}, p<0.0001, n=21). This was also true in RA patients (10.9 [3.6-29.9] fold increase migration of B10+ vs 4.8 [2.1-7.7] fold increase migration of B10^{neg}, p=0.009, n=12). SF from RA patients induced a significant migration of B10+ cells in CTL (7.3-fold increase [4.1-21.7], p=0.004, n=9) and RA patients (5.7-fold increase [2.3-7.9], p=0.008, n=10). This migration was correlated with the levels of CXCL13 in these SF, in CTL (r=0.7, p=0.05, n=9) but not in RA patients (n=10). Lastly, CXCL13 was also found to increase IL-10 secretion in B cells stimulated with CpG in CTL (1.5-fold increase [1.3-1.5], p=0.0002, n=13) and in RA patients (1.2-fold increase [1.1-1.3], p=0.005, n=12).

Conclusion: We showed that CXCL13 is a key driver for migration and differentiation of B10+ cells in CTL and in RA patients. However, the migration of B10+ cells in RA patients was not correlated with the level of CXCL13 in SF from RA patients, suggesting the implication of other CK in the migration of B10+ cells in RA.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.3544

THU0051 LOW-DOSE INTERLEUKIN-2 SELECTIVELY EXPAND AND ACTIVATE REGULATORY T CELLS ACROSS 13 AUTOIMMUNE DISEASES.

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Background: Regulatory T cells (Tregs) prevent autoimmunity and control inflammation. As low-dose interleukin-2 (ld-IL2) expands and activates Tregs, it has a broad therapeutic potential for any autoimmune or inflammatory disease (AID). We performed a disease-finding "basket trial" (TRANSREG NCT01988506) in patients affected by one of 11 different AID and reported the outcome of the first 46 patients (Rosenzweig et al, *ARD* 2019).

Objectives: Here we analyzed and discussed results from deep immunophenotyping, of 78 patients, to comprehensively study the effect of ld-IL2 on the immune system of patients affected by various AID

Methods: We performed a prospective, open label, phase I-IIa study in 78 patients with a mild to moderate form of one of 13 selected AID. All patients received ld-IL2 (1 million IU/day) for 5 days, followed by fortnightly injections for 6 months. Deep immunophenotyping was performed before and after 5 days of ld-IL2.

Results: ld-IL2 significantly expands both memory Tregs as well as naïve Tregs, including recent thymic emigrant Tregs. It also activates Tregs as demonstrated by the significantly increased expression of HLA-DR, CD39, CD73, GITR, CTLA-4. Similar results were observed across the different AID.

Conclusion: ld-IL2 "universally" improves Treg fitness across 13 autoimmune and inflammatory disease.

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Disclosure of Interests: Michelle Rosenzweig: None declared, Roberta Lorenzon: None declared, Patrice cacoub: None declared, Fabien Pitoiset: