Previous studies have suggested that alveolar macrophages (AMs) and T cells are associated with the pathogenesis of ILD. Recently, it is reported that co-inhibitory 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 co-culture assay with peripheral naïve CD4+ T cells of healthy individuals.

**Results:** Several co-inhibitory 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 cell populations correlated differentially 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 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 co-inhibitory 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 CD4+ T cells in IIM-ILD might be key factors in the disease process. The evaluation of co-inhibitory molecules on BALF T cells could be clinically useful.


**THU0050**

**CXL13 IS A KEY DRIVER FOR MIGRATION AND DIFFERENTIATION OF REGULATORY B CELLS**

C. Bempenu1, K. Schreiber2, J. Mielle3, P. Corbeaux4, J. Morel1, C. Dainel3, R. Aud0,2; CHU and University of Montpellier, Rheumatology, Montpellier, France; 2CNSR IGM UMR5553, Montpellier, France; 3Inmunology, Nimes, France

**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 (CR) 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 naive T cells towards regulatory T cells.

**Objectives:** Therefore, the aims of our study were to first characterize the profile of CR 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 Ficol 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 CR between B10+ and B10- cells from CTL and RA patients by flow cytometry. For migration assay, B10+ and B10- cells were sorted by FACSAria. Their ability to migrate on CRK found to ligand of CXCL13 was characterized in the first part (CCL21, CCL22, CCLX11, CXCL12 or CXCL13) or in soluble fluid (SF) from RA patients, were evaluated by migration assay in SmTranswell chambers and expressed as fold increase compared to basal migration towards control media.

**Results:** B10+ cells expressed a different profile of CRK compared to B10- cells both in CTL and RA patients and these profiles differed between B10+ cells of CTL and RA patients. However, no CRK profile could phenotypically define B10+ cells. Of note, CXCR5 was under-expressed on B10+ cell surface compared to B10+ cells in CTL (75% [98.2-100] fold increase positive among B10+ vs 99.2% [98.4-99.4] positive cells among B10-, p=0.006, n=10) and also in RA patients (78.3% [70.8-82.3] vs 98.2% [96.9-99.5], p=0.008, n=8). Nevertheless, mRNA expression of CXCR5 was higher among B10+ versus B10- 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- from CTL (9.1[5.6-14.6] fold increase migration of B10+ vs B10- 5.2 [3.1-7.5] fold increase migration of B10+ 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+ 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 [1.3-15], 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
None declared, Selim Aractangi: None declared, Beatrice Banville Speakers bureau: Lilly, Novartis, Laurent Beaugeois: None declared, Francis Berenbaum Grant/research support from: TRB Chemedica (through institution), MSD (through institution), Pfizer (through institution), Consultant of: Novartis, MSD, Pfizer, Lilly, UCB, Abbvie, Roche, Servier, Sanofi-Aventis, Flexion Therapeutics, Expanscience, GSK, Biogen, Nordic, Sandoz, Regeneron, Gilead, Bone Therapeutics, Regularis, Peptinov, 4P Pharma, Paid instructor for: Sandoz, Speakers bureau: Novartis, MSD, Pfizer, Lilly, UCB, Abbvie, Roche, Servier, Sanofi-Aventis, Flexion Therapeutics, Expanscience, GSK, Biogen, Nordic, Sanofi, Regeneron, Gilead, Sandoz, Julien Champey; None declared, Olivier Chazouillères: None declared, Christophe Corpechot: None declared, Bruno Fautrel Grant/research support from: Abbvie, Lilly, MSD, Pfizer, Consultant of: Abbvie, Biogen, BMS, Boehringer Ingelheim, Celgene, Lilly, Janssen, Medac, MSD France, Nordic Pharma, Novartis, Pfizer, Roche, Sanofi Aventis, SOBI and UCB, Arsene Mekinian: None declared, Elodie Regnier: None declared, David Sandron: None declared, Joe-Elie Salen: None declared, Jérémie Sel-LAM: None declared, Philippe Selskis: None declared, David Klattzmann Consultant of: LTLQ Pharma

DOI: 10.1136/annrheumdis-2020-eular.2743

THU0052

RELATIONSHIP BETWEEN INTERFERON-Γ PRODUCING IMMUNOCOMPETENT CELLS AND DISEASE ACTIVITY IN ACUTE-ONSET STILL’S DISEASE

Y. Shimizu1, D. Kishida1, T. Ichikawa1, Y. Sekijima1, Shinshu University, School of Medicine, Department of Medicine (Neurology and Rheumatology), Matsumoto, Japan

Background: In the acute phase of adult-onset Still’s disease (AOSD), elevated levels of IFN-γ producing cells in immune and inflammatory tissues including liver are shown. Moreover, IFN-γ impacts on activating macrophages which play a crucial role in the pathogenesis of AOSD. Natural killer (NK) cells and T helper cells are in impacts on activating macrophages which play a crucial role in the pathogenesis of AOSD. Meanwhile, increased expression of IFN-γ in the acute phase of AOSD. γ-Expression in CD4+ T cells and NK cells in patients with AOSD.

Methods: Twenty-four patients in the acute phase of AOSD (active AOSD), 8 of them after treatment (remission), and 12 healthy controls (HC) were recruited in this study. Peripheral blood mononuclear cells and serum samples were provided from them for the experimental analysis. Flow cytometry was used for analyzing γ-Producing CD4+ T cells, CD4+ regulatory T cells (Tregs), NK cells, and their intracellular IFN-γ expression levels as well as suppression assay of Tregs. The serum concentration of interleukin-18 (IL-18) was measured using commercially available ELISA kit. Relationship between the analyzed data and clinical findings related to disease activity were statistically evaluated.

Results: IFN-γ expression in CD4+ T cells was significantly higher in active AOSD than in HC (p < 0.05). Tregs also significantly higher expressed IFN-γ in active AOSD than in HC (p < 0.0001); and moreover, Tregs were significantly impaired in their suppression ability (p < 0.05). In both CD4+ T cells and Tregs, expression of IFN-γ was significantly correlated with serum ferritin levels in active AOSD (p < 0.05). IFN-γ expression in CD4+ T cells was significantly higher in patients with splenomegaly than those without that (p < 0.05). The proportion of NK cells was significantly lower in active AOSD than in HC (p < 0.0005), whereas IFN-γ expression in NK cells was significantly higher in active AOSD than in HC (p < 0.0005). The number of NK cells and IFN-γ expressing NK cells had inverse relationship with serum ferritin levels in active AOSD (p < 0.05 and p < 0.005, respectively). Increased number of NK cells and their decreased expression of IFN-γ were significantly demonstrated in remission (p < 0.05). In the analyses of NK cell subsets, lower expression of IFN-γ in CD56dim NK cells was higher than that in CD56bright NK cells was significantly indicated in active AOSD than in HC (p < 0.05). In remission, IFN-γ expression was significantly decreased in CD56dim NK cells (p < 0.05) despite no significant recovery of that in CD56bright NK cells (p = 0.311). Meanwhile, increased expression of IFN-γ in CD56dim NK cells was demonstrated in only patients who were treated with biologics. Although serum levels of IL-18 were significantly higher in active AOSD than in remission and HC, however, they had no significant correlations with any analyzed data.

Conclusion: CD4+ T cells and NK cells promote IFN-γ expression in the acute phase of AOSD. Meanwhile, increased expression of IFN-γ in CD4+ T cells and decreased number of NK cells were correlated with serum ferritin levels, suggesting that they are indicators of disease activity. Furthermore, high disease activity may impact on the alteration of IFN-γ-producing balance in two distinct population of NK cells, and the plasticity of Tregs leading to defect in suppression ability.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1817

THU0053

CONTRIBUTION OF DEFECTIVE NON-APOPTOTIC FAS SIGNALING TO IMMUNE DYSREGULATION IN ABDOMINAL LYMPHOPROLIFERATIVE SYNDROME (ALPS)

I. Stanis1, T. Kalina2, G. Andreuix3, M. Boerries3, J. Janowska4, M. Fuentes4, M. Bakardjeva5, J. Raabe6, J. Neumann6, J. Shucty7, V. Benes7, R. Garcia8, J. Garcia9, P. Diez8, A. Catala8, B. Neven9, O. Netti9, P. Olbrich9, R. Voil9, L. Alisina9, L. Allende10, L. Gonzales-Granado10, J. Thiel11, N. Venhof11, R. Lorenzetti12, S. Unger12, M. Seidl13, D. Mielenz14, P. Schneider14, S. Ehi14, A. Rensing-Ehi14, C. Smulski14, M. Rizzi14,15, University Medical Center Freiburg, Department of Rheumatology and Clinical Immunology, Freiburg, Germany;2 Charles University Prague, Department of Paediatric Haematology and Oncology, Prague, Czech Republic;3 University Medical Center Freiburg, Institute of Medical Bioinformatics and System Medicine, Freiburg, Germany;4 Cancer Research Center, Universidad de Salamanca, Salamanca, Spain;5 European Molecular Biology Laboratory, Heidelberg, Germany;6 Institut de Recerca Hospital Sant Joan de Déu Barcelona, Barcelona, Spain;7 University Hospital Nöcker-Enfants Malades, Paris, France;8 Instituto de Biomedicina de Sevilla, Sevilla, Spain;9 Hospital Sant Joan de Déu Barcelona, Barcelona, Spain;10 Hospital Universitario 12 de Octubre, Department of Immunology, Barcelona, Spain;11 University Hospital 12 de Octubre, Department of Pediatrics, Barcelona, Spain;12 University Medical Center Freiburg, Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Freiburg, Germany;13 University Medical Center Freiburg, Department of Pathology, Freiburg, Germany;14 Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany;15 University of Lausanne, Lausanne, Switzerland;16 Consejo Nacional de Investigaciones Científicas y Técnicas, Medical Physics Department, San Carlos de Bariloche, Argentina

Background: ALPS patients show impaired generation of humoral memory for F independent antigens whereas they generate memory for self-antigens due to impaired FAS-dependent removal of autoreactive germinal center B cells. It is known that FAS signaling via caspase activation results in cell apoptosis. However, FAS ligation may also initiate or modulate non-apoptotic signaling as shown for example by its ability to activate NF-κB. Recent data implicate a regulatory role of FAS in the modulation of mTOR signaling in ALPS double-negative T cells. Moreover, a recently described C194V FAS mutation disturbs its post-translational modification leading to impaired apoptosis induction while non-apoptotic signaling is still intact. Consequently, C194V FAS protects from the autoimmune phenotype in the murine ALPS system. This supports the hypothesis that FAS may prevent autoimmune with other mechanisms than inducing apoptosis.

Objectives: We hypothesize that FAS mutations impair this modulatory signaling, leading to hyper-activation of B cells. Therefore we aim to investigate non-apoptotic FAS signaling in B cells derived from healthy individuals and ALPS patients.

Methods: We studied resting and activated B cells in ALPS patients in presence or absence of FAS ligand (anti-FAS) and compared them to FAS negative B cells. In line with these data germinal center B cells and plasmablast from secondary lymphoid organs of ALPS patients show hyperactive mTOR signaling pathway. We used mass cytometry to perform functional phenotyping of B cells isolated from secondary lymphoid organs. Proteomic studies were performed to identify potential signaling circuits and RNA sequencing to study the consequences of FAS signaling on B cell fate.

Results: In CD40l activated B cells, FAS signaling results in specific modulation of the mTOR signaling pathway. This modulation is absent in ALPS derived B cells. In line with these data germinal center B cells and plasmablast from secondary lymphoid organs of ALPS patients show hyperactive mTOR signaling pathway. Proteomic studies identify a circuit that links FAS to the phosphatase Pten via Daxx and the deubiquitinase USP7.

Conclusion: We describe a new role of FAS in the regulation of B cell activation. Defects in FAS signaling in ALPS contribute to dysregulation of the mTOR signaling pathway and disturbed B cell development.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5733

THU0054

NKR-358, A NOVEL IL-2 CONJUGATE, STIMULATES HIGH LEVELS OF REGULATORY T CELLS IN PATIENTS WITH SYSTEMIC LUPUS ERYthematosus

S. Siddhants1, C. Fanton1, N. Dixit1, L. Lu1, V. Chindalore2, R. Levin3, I. Diابت4, R. Furie5, J. Zalewsky6, B. Kotzin7, N. Kekate, San Francisco, United States of America;8Pinnacle Research, Anniston, United States of America;9Clinical Research of West Florida, Clearwater, United States of America;10Paramount Medical Research, Middleton Heights, United States of America;11Northwell Health, Great Neck, United States of America

Background: Impaired IL-2 production and dysfunction of regulatory T cells (Tregs) have been identified as key immunological defects leading to the breakdown of immune self-tolerance in SLE. Low-dose IL-2 can expand Tregs, but

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5733