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

C. Rempenault¹, K. Schreiber¹, J. Mielles², P. Corbeau³, J. Morel¹, C. Daen^{1,2}, R. Audo². ¹CHU and University of Montpellier, Rheumatology, Montpellier, France; ²CNRS IGMM UMR5535, Montpellier, France; ³Immunology, Nîmes, 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 (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.

M. Rosenzweig¹, R. Lorenzon², P. Cacoub³, F. Pitoiset⁴, S. Aractingi⁵, B. Banneville⁶, L. Beaugerie⁷, F. Berenbaum⁷, J. Champey⁷, O. Chazouilleres⁷, C. Corpechot⁷, B. Fautrel⁶, A. Mekinian⁷, E. Regnier⁷, D. Saadoun⁶, J. E. Salem⁶, J. Sellam⁷, P. Seksik⁷, D. Klatzmann¹. ¹Sorbonne Université, Biotherapy, Paris, France; ²Sorbonne Université, Paris, France; ³APHP, Internal Medicine and Clinical Immunology, Paris, France; ⁴Sorbonne Université, Paris, France; ⁵APHP, Paris, France; ⁶APHP, Paris, France; ⁷APHP, Paris, France

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 (AIID). We performed a disease-finding "basket trial" (TRANSREG NCT01988506) in patients affected by one of 11 different AIID 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 AIID

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 AIID. 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 AIID.

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

References:

- [1] Rosenzweig M#, Lorenzon R#, Cacoub P, Pham HP, Pitoiset F, El Soufi K, Ribet C, Bernard C, Aractingi S, Banneville B, Beaugerie L, Berenbaum F, Champey J, Chazouilleres O, Corpechot C, Fautrel B, Mekinian A, Regnier E, Saadoun D, Salem JE, Sellam J, Seksik P, Daguenel-Nguyen A, Doppler V, Mariau J, Vicaute E, Klatzmann D. Immunological and clinical effects of low-dose interleukin-2 across 11 autoimmune diseases in a single, open clinical trial. *Ann Rheum Dis*. 2019 Feb;78(2):209-217. doi: 10.1136/annrheumdis-2018-214229. Epub 2018 Nov 24.

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

None declared, Selim Aractingi: None declared, Beatrice Banneville Speakers bureau: Lilly, Novartis, Laurent Beaugerie: 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, Regulaxis, 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, Sandoz, Regeneron, Gilead, Sandoz, Julien Champey: None declared, Olivier Chazouilleres: 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 Saadoun: None declared, Joe-Elie Salem: None declared, Jérémie SEL-LAM: None declared, Philippe Seksik: None declared, David Klatzmann Consultant of: ILTOO Pharma

DOI: 10.1136/annrheumdis-2020-eular.2743

THU0052

RELATIONSHIP BETWEEN INTERFERON- γ -PRODUCING IMMUNOCOMPETENT CELLS AND DISEASE ACTIVITY IN ADULT-ONSET STILL'S DISEASE

Y. Shimojima¹, D. Kishida¹, T. Ichikawa¹, Y. Sekijima¹. ¹Shinshu 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 proinflammatory cytokines including interferon- γ (IFN- γ) 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 charge of secreting IFN- γ in the innate and adaptive immune systems of disease, respectively. However, the features of their IFN- γ -producing variation depending on disease activity are still uncertain in AOSD.

Objectives: We investigated characteristics of IFN- γ -producing 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 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 indicated higher expression of 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 ($p < 0.005$), 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 CD56^{bright} NK cells and higher than in CD56^{dim} NK cells were significantly indicated in active AOSD than HC ($p < 0.05$). In remission, IFN- γ expression was significantly decreased in CD56^{dim} NK cells ($p < 0.05$) despite no significant recovery of that in CD56^{bright} NK cells ($p = 0.311$). Meanwhile, increased expression of IFN- γ in CD56^{bright} 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 AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

J. Staniek¹, T. Kalina², G. Andrieux³, M. Boerries³, I. Janowska¹, M. Fuentes⁴, M. Bakardjieva², J. Raabe¹, J. Neumann¹, J. Stuchly², V. Benes⁵, R. Garcia⁴, J. Garcia⁴, P. Diez⁴, A. Catala⁶, B. Neven⁷, O. Neth⁸, P. Olbrich⁸, R. Voll¹, L. Alsina⁹, L. Allende¹⁰, L. Gonzales-Granado¹¹, J. Thiel¹, N. Venhoff¹, R. Lorenzetti¹, S. Unger¹², M. Seidl¹³, D. Mielenz¹⁴, P. Schneider¹⁵, S. Ehl¹², A. Rensing-Ehl¹², C. Smulski¹⁶, M. Rizzi^{1,12}. ¹University Medical Center Freiburg, Department of Rheumatology and Clinical Immunology, Freiburg, Germany; ²Charles University, Prague, Department of Paediatric Haematology and Oncology, Prague, Czech Republic; ³University Medical Center Freiburg, Institute of Medical Bioinformatics and System Medicine, Freiburg, Germany; ⁴Cancer Research Center, Universidad de Salamanca, Salamanca, Spain; ⁵European Molecular Biology Laboratory, Heidelberg, Germany; ⁶Institut de Recerca Hospital Sant Joan de Déu Barcelona, Barcelona, Spain; ⁷University Hospital Necker-Enfants Malades, Paris, France; ⁸Instituto de Biomedicina de Sevilla, Sevilla, Spain; ⁹Hospital Sant Joan de Déu Barcelona, Barcelona, Spain; ¹⁰Hospital Universitario 12 de Octubre, Department of Immunology, Barcelona, Spain; ¹¹University Hospital 12 de Octubre, Department of Pediatrics, Barcelona, Spain; ¹²University Medical Center Freiburg, Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Freiburg, Germany; ¹³University Medical Center Freiburg, Department of Pathology, Freiburg, Germany; ¹⁴Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany; ¹⁵University of Lausanne, Lausanne, Switzerland; ¹⁶Consejo Nacional de Investigaciones Científicas y Técnicas, Medical Physics Department, San Carlos de Bariloche, Argentina

Background: ALPS patients show impaired generation of humoral memory for T 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- κ B. 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 signalling is still intact. Consequently, C194V FAS protects from the autoimmune phenotype in the murine ALPS system. This supports the view that FAS may prevent autoimmunity 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 by flow cytometry analysing relevant molecules to the CD40 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

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

S. Siddhanti¹, C. Fanton¹, N. Dixit¹, L. Lu¹, V. Chindalore², R. Levin³, I. Diab⁴, R. Furie⁵, J. Zalevsky¹, B. Kotzin¹. ¹Nektar, San Francisco, United States of America; ²Pinnacle Research, Anniston, United States of America; ³Clinical Research of West Florida, Clearwater, United States of America; ⁴Paramount Medical Research, Middleburg Heights, United States of America; ⁵Northwell 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