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.

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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.

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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 (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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