integrating single B cell expression profiling and repertoire analysis, we map the development of B cells in BM and peripheral and pathogenic characteristics of early B cells, especially pre-B.

Conclusion: These findings demonstrated that early B cells in BM, especially pre-B are abnormally differentiated with dysregulations, BM is an important organ targeted by SLE. This study not only to clarify the internal mechanism of the disorder of differentiation of B cells, but also to provide new clues for the targeted diagnosis and treatment of SLE.

References:

Methods: A centromere protein library was created by cloning 6 single proteins specific to centromere antigens. The aim of this study is to elucidate centromere autoantigens comprehensively and clarify their association with pathogenesis of SjS, SSc and PBC.

Results: Patients had a wide variety of antibodies against most of centromere antigens including 4 newly identified autoantigens. The hierarchical clustering of each antigen distinguished 2 antigen clusters. The reactivity of autoantibodies to each centromere protein was analyzed by flow cytometry.

Conclusion: We identified 4 novel centromere autoantigens and our data suggested that the target of ACA was the protein complex rather than a single specific antigen in SjS, SSc and PBC patients. Using the combination of centromere proteins may be useful to detect ACA with higher sensitivity.

Disclosure of Interests: Nobuhiko Kajo: None declared, Masaru Takeshita: None declared, Katsuya Suzuki: 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., Danippon Sumitomo PLC, None declared, K. Chemin1, V. Malmström1, 1Karolinska Institutet, Division of Rheumatology, Department of Medicine, Solna, Sweden Background: Autoimmunity to citrullinated autoantigens forms a critical component of disease pathogenesis in rheumatoid arthritis (RA). Presence of anti-citrullinated protein antibodies (ACPA) in patients has high diagnostic value. Recently, several citrullinated antigen specific CD4+T cells have been described. However, detailed studies of their T-cell receptor usage and in vivo profile suffer from the disadvantage that these cells are present at very low frequencies. In this context, we here present a pipeline for TCR repertoire analysis of antigen-specific CD4+T cells from RA patients, including both citrulline and influenza (control) specificities using in vitro peptide challenge induced-cell expansion.

Objectives: To enable studies of the T cell repertoire of citrullinated antigen-specific CD4+T cells in rheumatoid arthritis

Methods: Peripheral blood mononuclear cells (PBMCs) (n=7) and synovial fluid mononuclear cells (SFMCs) (n=5) from HLA-DR*0401-positive RA patients were cultured in the presence of citrullinated Tenas cin C peptide cocktails or influenza peptides (positive control). Citrulline reactive cells were further supplemented with recombinant human IL-15 and IL-7 on day 2. All cultures were replenished with fresh medium on day 6 and rIL-2 was added every 2 days from then. Assessment of proportion of peptide-HLA-tetramer positive cells was performed using flow cytometry whereby individual antigen-specific CD4+ T cells were sorted into 96-well plates containing cell lysis buffer, followed by PCR -based alpha/beta TCR sequencing. TCR sequencing data was demultiplexed and aligned for TCR gene usage using ImmuSEQ. Some tetramer positive cells were sorted into complete medium containing human IL-2 and PHA for expansion of antigen-specific cells. Cells were supplemented with irradiated allogeneic PBMCs (30 times number of antigen specific cells). Clones of antigen specific CD4+ T cells were further subjected to tetramer staining to confirm expansion of cells.

Results: As evidenced by increase in frequency of tetramer positive CD4+ T cells, in vitro peptide stimulation resulted in expansion of both influenza specific (Fig. 1a) and citrullinated antigen specific (Fig. 1b) CD4+T cells. Polyclonal in vitro expansion of tenasin C tetramer positive sorted cells followed by tetramer staining further confirmed antigen specificity and enrichment for antigen specific CD4+T cells after polyclonal stimulation (Fig.1c). TCR repertoire analysis in PB and SF dataset from the first patient showed clonal expansion of influenza specific cells in both sites. Synovial fluid had more diversity of expanding clones as compared to paired PB, with few expanded clones being shared among SF and PB. We observed a more diverse TCR repertoire in citrulline specific CD4+T cells. We also observed sharing of TCR alpha chains among different citrulline specific CD4+T cell clones.

Conclusion: This method provides a highly suitable approach for investigating TCR specificities of antigen specific CD4+T cells under conditions of low yields. Building on this dataset will allow us to assess specific features of TCR usage of autoreactive T cells in RA.

PBMCs were cultured in presence of (a) influenza (HA, MP54) and (b) citrullinated tenasin peptides. The proportion of antigen specific CD4+T cells was assessed using HLA-class II tetramer staining. We observed an increase in frequency of (a) Influenza specific cells (red dots in upper left and lower right quadrants) and (b) citrullinated tenasin C specific cells (red dots in lower right quadrant), at day 13 post culture as compared to day 3. (c) Sorting of citrullinated
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

J. Y. Leong1, P. K. Kumar1, G. Mijnheer2, P. Chen1, J. G. Yeo1,3, S. H. Tay1, C. Chua1, S. N. Hazirah1, L. Lal1, A. Consolo1, M. Gattorno1, T. Arkachaisri1,2, A. Martins1, F. Van Wijk1, S. J. Alibani1,3. 

1Translational Immunology Institute, SingHealth/Duke-NUS Medical Academic Centre, Singapore, Singapore; 
2Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands; 
3Division of Medicine, KK Women’s and Children’s Hospital, Singapore, Singapore; 
4Second Paediatric Division, University of Genova and G Gaslini Institute, Genova, Italy

**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 utilized 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, correlating with disease activity and overlap in synovial TCR repertoire. A similar immunation-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 ontology 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 SFMCs and examined by CyTOF.

**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/Itregs. This immunological relationship between CPLs/iaTregs is reaffirmed by comparative differential gene expression (DEG) and phylogenetic tree analysis, which indicated transcriptional convergence between circulatory pathogenic CPLs/iaTregs subsets and divergence from their respective conventional Teff/Itreg 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 pathogenetic 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/iaTregs and conventional Teff/Itreg subsets from (a) healthy PBMCs, (b) JIA PBMCs and (c) paired JIA SFMCs. The convergence between CPLs/iaTregs 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.

**References:**


