Background:

Previously been documented. The number and the extent of organisation of B cells in RA has been described in the peripheral blood of patients with seropositive RA. The role of these B cells is still not fully understood.

Methods: Peripheral blood was drawn from seropositive RA patients with an incomplete response to csDMARDs (n=29) who initiated biological therapy with TNF blockers (TNFb) (n= 17) or abatacept (n= 12), prescribed based on routine clinical practice. cTfh and Tph cell frequencies were determined by flow cytometry of freshly isolated PBMCs at the basal visit and 6 months after starting treatment escalation. For each patient, an age and gender-matched healthy control (HC) was also studied at both time points (n=29).

Results: As compared with HC, active RA patients receiving csDMARDs demonstrated a baseline increased frequency of both cTfh and Tph cells. A significant improvement of disease activity as determined by the DAS28 score was apparent in all of the patients 6 months after initiating biologicals. At that time point, a significant reduction of the previously elevated cTfh cell frequency was observed in both treatment groups. However, cTfh cells remained elevated in patients receiving TNFb notwithstanding a good therapeutic response, whereas subjects receiving abatacept experienced a significant abatement of their cTfh cell frequency. Experimental variation of the cTfh and Tph cell numbers in HC was minimal.

Conclusion: Abatacept but not TNFb, are able to bring down cTfh cell numbers in RA. This indicates that costimulation blockade can help attain an immunological remission, whereas TNF neutralization may allow a persistent pathogenic germinal center overactivity. At the same time, treatment with both abatacept and TNFb results in a downmodulation of the previously elevated cTfh cell numbers, in parallel with the remitting local joint inflammation.

References:


Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.4435

THU0044

SINGLE CELL ANALYSIS OF BONE MARROW AND PERIPHERAL ALTERED B CELL DIFFERENTIATION IN PATIENTS WITH ACTIVE SLE AND THE MECHANISM OF ABNORMAL EARLY B CELL DEVELOPMENT

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Background: B cell differentiation and dysfunction play a key role in the pathogenesis of Systemic lupus erythematosus (SLE). Bone marrow (BM) is the development organ of B cells, and also the home and residence place of plasma cells and memory B cells. However, there is a lack of studies on B cells in BM with lupus.

Objectives: To map the development of BM and peripheral B cells and investigate the mechanism of abnormal early B cell development in SLE.

Methods: A total of 11 SLE patients and 5 age- and sex-matched controls were recruited. BM and peripheral B cell subsets were measured by flow cytometry, sorting-purified B cell subsets were subject to Single-cell RNA sequencing (scRNA-seq) and functional studies. Plasma cytokines and secreted immunoglobulins were detected by Luminox or ELISA. Disease activity of SLE patients was measured using the SLE Disease Activity Index (SLEDAI).

Results: In the present study, we find out that the percentage of monocytes in MNC (p=0.070) and plasma cells (p=0.001) in CD19 + were significantly decreased in BM of SLE, compared to healthy controls. While SLE patients had increased T%MNC (p=0.008) and B%CD19 (p=0.002) in BM that controls. In detail, the B cells subsets of bone marrow in patients with active lupus (SLEDAI>8 score) were seriously disordered, showing the increasing T%MNC (p=0.049), B%CD19 (p=0.006) and immature B cell%CD19 (p=0.010) than healthy donors, B%CD19+ exhibited good relationship with SLEDAI.
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 pro-B.

Conclusion: These findings demonstrated that early B cells in BM, especially pro-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:

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.6162

THU0045

IDENTIFICATION OF NOVEL CENTROMERE AUTOANTIGENS IN SJÖGREN’S SYNDROME, SYSTEMIC SCLEROSIS AND PRIMARY BILIARY CHOLANGITIS.

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Background: Anti-centromere antibodies (ACA) are detected in the serum of patients with various autoimmune diseases including Sjögren’s syndrome (SjS), systemic sclerosis (SSc) and primary biliary cholangitis (PBC). ACA positivity is correlated with clinical manifestations such as Raynaud’s phenomenon and sclerodactyly and these features are commonly seen across diseases. Although CENP-Protein is thought to be the major antigen against ACA, autoimmune features of other centromere proteins have not been fully evaluated.

Objectives: The aim of this study is to elucidate centromere autoantigens comprehensively and clarify their association with pathogenesis of SjS, SSc and PBC.

Methods: A centromere protein library was created by cloning 6 single proteins and 10 complexes consisting of 35 proteins belonging to human centromere region. The centromere antigens were immobilized on beads and incubated in the serum of patients with SjS (n = 86), SSc (n = 35), PBC (n = 10), patients with two or more diseases above (n = 44), and healthy volunteers (n = 68). Autoantibodies to each centromere protein were analyzed by flow cytometry.

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 against a centromere protein of one cluster was mutually correlated regardless of disease types, indicating that these proteins/protein complexes might be the target of ACA. In addition, our method enabled us to detect sera reacted against multiple centromere antigens in some of the ACA-negative patients with existing methods.

Conclusion: We identified 4 novel centromere autoantigens and our data suggested that the main 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.

References:

Disclosure of Interests: Nobuhiko Kajio: None declared, Masaru Takeshita: None declared, Katsuya Suzuki: None declared, Tsutomu Takeuchi Grant/research support from: Eisai Co., Ltd, Astellas Pharma Inc., AbbVie GI, Asta

THU0046

A PIPELINE TO STUDY ANTIGEN-SPECIFIC CD4+ T CELLS IN RHEUMATOID ARTHRITIS

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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 citrullin antibody 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 citrullin 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=71) and synovial fluid mononuclear cells (SFMCs) (n=5) from HLA-DQª0401-positive RA patients were cultured in the presence of citrullinated Tenascin 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 replated with fresh medium on day 6 and rIL-2 was added every 2 days from then. Assesment 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 PCRs-based alpha/beta TCR sequencing. TCR sequencing data was demultiplexed and aligned for TCR gene usage using MiXCR. 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 tenascin 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 tenascin 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 tenascin C specific cells (red dots in lower right quadrant), at day 13 post culture as compared to day 3. (c) Sorting of citrullinated

Fig. 1 In-vitro expansion of antigen specific CD4+T cells: