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 prope-B.

Conclusion: These findings demonstrated that early B cells in BM, especially prope-B are abnormally differentiated with dysregulations. BM is an important organ targeted by SLE. This study is 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.

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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 CENPB 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.

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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 (ACPAs) 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 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 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 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 allogenic 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.

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

Conclusion: This method provides a highly suitable approach for investigating TCR specificities of antigen specific CD4+T cells under conditions of low cell 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