References:

- [1] Berglund, L.J., Avery, D.T., Ma, C.S., Moens, L., Deenick, E.K., Bustamante, J., Boisson-Dupuis, S., Wong, M., Adelstein, S., Arkwright, P.D., et al. (2013). IL-21 signalling via STAT3 primes human naive B cells to respond to IL-2 to enhance their differentiation into plasmablasts. *Blood* 122, 3940–3950.
- [2] Menon, M., Blair, P.A., Isenberg, D.A., and Mauri, C. (2016). A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus. *Immunity* 44, 683–697.

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THU0042

DIFFERENTIAL EFFECT OF ABATACEPT VS TNF BLOCKERS, ON THE FREQUENCY OF CIRCULATING FOLLICULAR HELPER (TFH) AND PERIPHERAL HELPER (TPH) T CELLS IN RHEUMATOID ARTHRITIS

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Background: CXCR5+PD-1^{hi} follicular helper (Tfh) and CXCR5+PD-1^{hi} peripheral helper (Tph) T cells play an important role in the pathogenesis of Rheumatoid Arthritis (RA) by providing help to autoantibody secreting B cells. Whereas Tfh cells typically dwell in the germinal centers of lymphoid organs, Tph cells accumulate at inflamed tissues. An increased frequency of Tph cells and of circulating counterparts of Tfh cells have been described in the peripheral blood of patients with seropositive RA.

Objectives: To examine the effect of treatment escalation using biological agents (TNF blockers or abatacept), on the frequency of circulating Tfh (cTfh) and Tph (cTph) cells in RA.

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 cTph 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 cTph cells. A significant improvement of disease activity as determined by the DAS28 score (Δ DAS28>2.0) was apparent in all of the patients 6 months after initiating biologicals. At that time point, a significant reduction of the previously elevated cTph 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 cTph 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 TNF blockers results in a downmodulation of the previously elevated cTph cell numbers, in parallel with the remitting local joint inflammation.

References:

- [1] Simpson N et al, Arthritis Rheum 2010; Craft J, Nat Rev Rheumatol 2012; Arroyo-Villa I et al., Arthritis Res Ther 2014; Rao DA et al., Nature 2017.

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THU0043

EXPRESSION OF B CELL CHEMOKINE-CHEMOKINE RECEPTOR PATHWAYS IS ALTERED IN GIANT CELL ARTERITIS

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Background: The presence of organised B cells in both cranial-giant cell arteritis (C-GCA) (temporal artery) and large vessel (LV)-GCA (aorta) has previously been documented. The number and the extent of organisation of B cells

in tertiary lymphoid organs (TLO) was more prominent in the aorta than in the temporal artery, suggesting possible differences in B cell phenotype, kinetics and tropism between C-GCA and LV-GCA.

Objectives: We sought to analyse B cell differentiation subsets in both C-GCA and LV-GCA and to investigate differences in the expression of chemokine pathways involved in B cell migration and TLO organisation.

Methods: Blood was collected from C-GCA (n=11) and LV-GCA (n=22) patients at baseline, before start of glucocorticoid treatment, and after 3 months of treatment. The LV-GCA groups consisted of 11 patients with isolated LV-GCA and 11 patients with overlap LV/C-GCA. Also, age- and sex- matched healthy controls (HC, n=24) were included. The following chemokines were measured with Luminex in the sera of patients and HC: BAFF, CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL12, and CXCL13. Thawed PBMC of 7 C-GCA, 10 LV-GCA and 24 HC were stained with antibodies against CD19, CD27, IgD, IgM, CD38, CXCR3, CXCR4, CXCR5, and CCR7 to allow identification of B cell differentiation subsets and their chemokine receptor expression.

Results: We found a lower absolute number of CXCR3+ memory and double negative (late stage) B cells in GCA patients when compared to healthy controls. Also, the absolute number of CXCR5+ memory B cells was lower in patients than in controls. Chemokine receptor expression on circulating B cells did not significantly differ between C-GCA and LV-GCA at baseline. After 3 months of treatment, frequencies and absolute numbers of both CXCR3+ and CXCR5+ memory B cells increased. In sera of all GCA patients, CXCL9 (which is a chemokine involved in migration of B cells to sites of inflammation) and CXCL13 (which is involved in local organization of B cells) were significantly increased. BAFF and CCL21 were increased only in LV-GCA when compared to HC. Serum chemokine levels did not differ between C-GCA and LV-GCA patients. An inverse correlation was observed between B cell counts and CXCL9 as well as CXCL13 in LV-GCA, only. After 3 months of treatment, CXCL9 levels remained elevated whereas CXCL13 increased even further.

Conclusion: At diagnosis, CXCL9 and CXCL13 were significantly increased in all GCA patients as compared to HC. Elevated CXCL9 levels inversely correlated with B cells numbers in LV-GCA, only, which may suggest that B cells preferentially migrate to the inflamed aorta via a mechanism involving CXCL9. In addition, CXCL13 may be linked to local TLO organization in LV-GCA. Currently, we are studying the local expression of chemokines and chemokine receptors at the site of inflammation in both C- and LV-GCA.

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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 Luminex 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 than controls. In detail, the B cell subsets of bone marrow in patients with active lupus (SLEDAI≥8 score) were seriously disordered, showing the increasing T%MNC (p=0.049), prope-B%CD19+ (p=0.006) and immature B cell%CD19+ (p=0.010) than healthy donors. prope-B%CD19+ exhibited good relationship with SLEDAI. By

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.

References:

- Palanichamy, A., et al., *Neutrophil-mediated IFN activation in the bone marrow alters B cell development in human and murine systemic lupus erythematosus*. J Immunol, 2014. **192**(3): p. 906-18.
- Papadaki, H.A., J.C. Marsh, and G.D. Eliopoulos, *Bone marrow stem cells and stromal cells in autoimmune cytopenias*. Leuk Lymphoma, 2002. **43**(4): p. 753-60.
- Karrar, S. and D.S. Cunningham-Graham, *Abnormal B Cell Development in Systemic Lupus Erythematosus: What the Genetics Tell Us*. Arthritis Rheumatol, 2018. **70**(4): p. 496-507.
- Woods, M., Y.R. Zou, and A. Davidson, *Defects in Germinal Center Selection in SLE*. Front Immunol, 2015. **6**: p. 425.
- Upregulation of p16INK4A promotes cellular senescence of bone marrow-derived mesenchymal stem cells from systemic lupus erythematosus patients. Cell Signal, 2012. **24**(12): p. 2307-14.

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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.

References:

- Fritzler MJ, Rattner JB, Luft LM, Edworthy SM, Casiano CA, Peebles C, Mahler M. Historical perspectives on the discovery and elucidation of autoantibodies to centromere proteins (CENP) and the emerging importance of antibodies to CENP-F. Autoimmun Rev. 2011;10:194-200.

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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.

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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