Conclusion: The results further support the involvement of monocytes in RA pathogenesis and highlight the key role MOSPD2 plays in this disease. Accordingly, targeting of monocyte migration using anti MOSPD2 mAbs may hold promise as a treatment for various chronic inflammatory diseases, including RA.

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FRI0008

IGM ANTIBODIES AGAINST MALONDIALDEHYDE AND PHOSPHORYLCHOLINE IN DIFFERENT SYSTEMIC RHEUMATIC DISEASES

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Background: IgM antibodies against phosphorylcholine (anti-PC) and malondialdehyde (anti-MDA) may have protective properties in both atherosclerosis and rheumatic disease, especially anti-PC. Low levels of IgM anti-PC is associated with SLE itself and also with atherosclerotic plaques¹ and with being a non-responder to biologics in RA.¹ We determined mechanisms by which anti-PC (and to some extent anti-MDA) could be protective: 1: anti-inflammatory; 2: inhibition of uptake of oxLDL in macrophages; 3: inhibition of cell death.¹ 4: increase in clearance of human dead cells.² 5: anti-PC promotes polarization of T regulatory cells in SLE-patients' T cells from a low level and also in cells from atherosclerotic plaques.³

Objectives: To compare systemic rheumatic diseases in relation to natural anti-PC and anti-MDA, to develop novel classifications but also potential treatment against rheumatic disease. We here determine anti-PC and anti-MDA in different systemic rheumatic conditions and study their potential role properties.

Methods: Anti-PC and anti-MDA was measured using ELISA in patients with SLE (374), RA (354), Mixed connective tissue disease (MCTD, 77), Systemic sclerosis (SSc, 331), Sjögren's syndrome (SjS, 324), primary antiphospholipid syndrome (PAPs, 65), undifferentiated connective tissue disease (UCTD, 118) and 515 matched healthy controls (HC). Cardiovascular score (CV) was broadly defined based on clinical disease symptoms. Anti-PC and anti-MDA peptide/protein characterization were compared using a proteomics de novo sequencing approach. anti-MDA and anti-PC were extracted from total IgM. The proportion of Treg cells was determined by flow cytometry.

Results: The maximal difference between cases and controls was shown for MCTD: significantly lower IgM Anti-PC but not anti-MDA among patients (median 49.3RU/ml vs 70.4 in healthy controls, p(t-test)=0.0037). IgM low levels were more prevalent in MCTD, SLE, SjS, SSc and UCTD. IgM anti-PC variable region profiles were different from and more homologous than anti-MDA. Anti-PC but

not anti-MDA were significantly negatively correlated with CV in the whole patient group. In contrast to IgM anti-PC, anti-MDA did not promote polarization of Tregs. **Conclusion:** Anti-PC is decreased in MCTD and also in SLE, SjS and SSc but not in other studied diseases. Anti-PC may thus differentiate between these. In contrast, anti-MDA did not show these differences between diseases studied. Anti-PC level is negatively correlated with CV in the patient group cohort. In contrast to anti-PC, anti-MDA did not promote Treg polarization. These findings could have both diagnostic and therapeutic implications, one possibility being active or passive immunization with PC in some rheumatic conditions.

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FRI0009

IMMUNO-PHENOTYPIC ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN RHEUMATOID ARTHRITIS PATIENTS TREATED WITH E6011, A HUMANIZED ANTI-FRACKALKINE MONOCLONAL ANTIBODY

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Background: Fractalkine (FKN) and its solo receptor CX3CR1 are deeply involved in the pathogenesis of rheumatoid arthritis (RA). FKN is expressed on vascular endothelium, while CX3CR1 is expressed on peripheral blood leukocytes such as monocytes, macrophages, NK cells, effector CD8⁺ T cells and a minor fraction of CD4⁺ T cells. E6011, a novel humanized anti-FKN monoclonal antibody (mAb), is under clinical development in RA.

Objectives: In order to continuously assess the E6011 pharmacodynamics by monitoring the alteration of peripheral blood immune cells, including CX3CR1-expressed cell populations, a series of multi-color flow cytometry (FCM) was conducted before and during the course of the E6011 treatment of active RA patients in phase 2 clinical trial.

Methods: Immuno-phenotypic changes were explored by FCM during the E6011 administration in 190 Japanese RA patients with inadequate response to MTX (NCT02960438). Patient's peripheral blood were drawn into fixative tube (Cytocheck® BCT, Streck) at each clinic and thereafter transported to the FCM facility at KAN Research Institute, Inc. within 30 hours after the blood collection to operate the FCM analysis by standardized method. Immuno-phenotyping was carried out by multi colors flow cytometry (BD FACSCanto™, BD LSRFortessa™, BD Biosciences).

Results: Based on these determined conditions, CX3CR1 expression on monocytes, NK cells and a part of CD8⁺ and CD4⁺ T cells were confirmed in this method. Interestingly, during the E6011 treatment, the proportion of CD16⁺ monocytes, which highly express CX3CR1 within whole monocytes, were significantly decreased at 2 week after initial treatment from the baseline (E6011: p < 0.001, placebo: p > 0.48) and sustained up to 24 week, while that of CD16⁺ monocytes were increased. The reduction of the frequency of CX3CR1⁺ cells in NK cells, CD4⁺ and CD8⁺ T cells were not observed, but in some certain populations like CX3CR1-expressed CD56⁺CD16⁺ NK cells and terminal differentiated effector CD8⁺ T cells, the frequency of CX3CR1⁺ cells in these populations tended to increase from the baseline at 2 week and kept increasing up to 24 week by the E6011 treatment.

Conclusion: E6011 significantly decreased the proportion of CD16⁺ monocytes in whole monocytes. Our results indicated that the reduction of CD16⁺ monocytes after initial treatment might be a sensitive marker of E6011 in peripheral blood,

possibly reflecting mechanism of action of E6011, since the CD16⁺ monocytes highly express CX3CR1.

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FRI0010

GM-CSFR PATHWAY IS IMPLICATED IN PATHOGENIC INFLAMMATORY MECHANISMS IN GIANT CELL ARTERITIS

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Background: Giant Cell Arteritis (GCA) is characterized by inflammation of large and medium arteries. Classic symptoms include headaches, malaise and, in severe cases, blindness and aortic aneurysms. Corticosteroids (CS) are the first line of treatment. Relapsing disease patients undergo multiple courses of CS therapy increasing their CS exposure and toxicity. A significant unmet need for disease-modifying CS-sparing therapy remains in GCA as the efficacy of current treatment options, including tocilizumab have limitations.

We have previously reported elevated expression of granulocyte-macrophage colony stimulating factor (GM-CSF) pathway transcriptomic signature in GCA vessels. GM-CSF may contribute to underlying disease mechanisms by regulating inflammatory macrophages, dendritic cells (DCs) and T helper (T_H1/T_H17) cells which are involved in GCA pathogenesis. GM-CSF produced by T cells¹ can promote polarization of inflammatory macrophages² and recruitment and differentiation of monocytes into inflammatory DCs² that can in turn recruit T cells and stimulate T_H1/T_H17 differentiation creating a feedback loop. GM-CSF may also exert direct effects on angiogenesis³ and vessel wall remodeling⁴.

Objectives: To demonstrate the contributing role of GM-CSF pathway to inflammation in GCA arteries.

Methods: Immunostaining was used to examine expression of GM-CSF and GM-CSF-Rα proteins in temporal artery biopsies (TABs) from GCA and controls (patients with suspected but not confirmed GCA and a negative TAB). Costaining with cell markers such as CD31, CD3, and CD68 allowed visualization of cells expressing GM-CSF and GM-CSF-Rα. Expression of GM-CSF pathway molecules such as phospho-JAK2 and PU.1 proteins was detected by immunohistochemical staining of GCA and control TABs. *Ex vivo* cultured GCA arteries treated (10 each) with mavrilimumab (anti-GM-CSF-Rα) or placebo for 5 days were assayed for gene expression by qPCR, and culture supernatants were analyzed by ELISA.

Results: Endothelial cells and macrophages were the main cell types expressing GM-CSF and GM-CSF-Rα. Increased expression of phospho-JAK2 (activated signaling molecule) and nuclear-localized PU.1 (transcription factor) in GCA TABs compared to controls indicated the presence of active GM-CSF signaling pathway in GCA.

Inhibition of PU.1 mRNA expression in *ex vivo* cultures of GCA arteries treated with mavrilimumab indicated blockade of GM-CSFR signaling pathway. Mavrilimumab induced decrease in mRNA expression of key cell type markers including DC and macrophage activation markers CD83 and HLA-DRA, monocyte markers CD14 and CD16, T cell marker CD3ε, and B cell marker CD20 in these GCA artery cultures. Expression of inflammatory T_H1/T_H17 factors IFNγ (mRNA), TNFα, CXCL10 (IFNγ-stimulated chemokine) and IL-6 (mRNA and protein) was also inhibited by mavrilimumab in GCA artery cultures.

Conclusion: Increased GM-CSF, GM-CSF-Rα, and downstream pathway-associated protein levels in GCA biopsies were consistent with previously-observed increased transcriptome signature. Expression of genes associated with inflammatory cells was suppressed by mavrilimumab in cultured GCA arteries. These data implicate the GM-CSF pathway in GCA pathophysiology and increase confidence in rationale for targeting the GM-CSF pathway in GCA.

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FRI0011

DEVELOPMENT OF A HIGH-DIMENSIONAL FLOW CYTOMETRY PANEL TO ANALYSE NATURAL KILLER CELLS IN SLE

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Background: Natural Killer (NK) cells are an innate immune cell type that has somewhat been overlooked in the context of systemic lupus erythematosus (SLE). SLE patients display a reduced number of NK cells with an activated phenotype and increased capacity to produce IFN-γ, decreased antibody-dependent cellular cytotoxicity (ADCC), and altered natural cytotoxicity (1). NK cell activation is determined by the integration of input from a myriad of activating and inhibitory receptors. Previously, using Nanostring® gene expression technologies, we found our SLE cohort showed decreased gene expression of a number of these receptors (KLRC2, KLRC1, KLRB1, KLRF1, KLRG1, PRF1 and IL2RB) leading us to explore NK cells in SLE in more depth.

Objectives: Our aim was to develop a high-dimensional flow cytometry panel to characterise dysregulation of NK cell in SLE, with particular reference to the activating and inhibitory receptors found to be dysregulated in SLE at the gene expression level.

Methods: Markers for NK panel were selected to include canonical phenotyping/functional molecules of NK cells with a particular emphasis on receptors found to be lower in our SLE cohort's gene expression findings. NK panel was designed to minimise spectral overlap, expression and co-expression of markers was taken into consideration. Antibodies were titrated, and voltages optimised to achieve the best separation index for each of the antibodies. The 24-marker panel was run on 52 SLE patients of various disease manifestations, treatments and disease severity. 20 healthy controls were also run for comparison.

Results: A 24-marker flow cytometry panel including 19 NK cell antigens was optimised, including basic phenotype (CD3/CD56/CD16/NKp46) and NK differentiation markers (CD57/CD94), activating and inhibitory receptors (NKG2A/NKG2C/NKG2D), costimulatory receptors (CD244/CD226), transcription factors (Eomes/Tbet) and effector molecules (granzyme/perforin). Immunophenotypic high-parameter analysis of SLE and control samples is in progress and results will be presented.

Conclusion: Our development of a high-dimensional immunophenotypic panel allows identification of changes in NK cells in SLE including antigen expression levels, subset percentages and potentially of novel subsets. This panel will be used to investigate NK cell changes with disease course/activity, therapeutic response, and to discover potential drug targets for SLE.

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