Objectives: The purpose was to detect the susceptibility genes of RP through whole-exome sequencing (WES) in a Chinese family and deepen our understanding of the pathogenesis of RP.

Methods: A 32 year-old Chinese female proband with RP and her family in which only her mother was RP patient were recruited in the current study. The genomic DNA of 6 human subjects was extracted from the peripheral blood monocyte cells (PBMCs) and then identified gene allele mutations using WES. Candidate variants with low frequency (<0.1%) in general population and predicted deleterious on gene function were identified. Sanger sequencing was then used to validate the analysis results of WES and further validated the gene variants in 12 human subjects.

Results: 38 genes mutated were confirmed by WES among RP patients. Of them, 10 gene mutated were validated by Sanger sequencing, including Collagen Type XXII Alpha 1 Chain (COL22A1) rs200464636, folliculin (FLCN) NM_144606: c.G838A: p.E280K, glycosylphosphatidylinositol anchor attachment 1 (GPAA1) rs201424010, DNA ligase 3 (LIG3) rs761808558, RecQ like helicase 4 (RECQL4) rs757703895, ring finger protein 207 (RNF207) NM_207396: c.T425C: p.I142T, coiled-coil domain containing 61 (CCDC61) rs777816675, Purkinje cell protein 2 (PCP2) rs144974437, tubulin alpha 3e (TUBA3E) rs749780020 and myosin heavy chain 15 (MYH15) NM_014981: c.G4462A: p.A1488T.

Conclusions: This study confirms that coinheritance of multigene mutated may contribute to the susceptibility to RP. The candidate genes mutated we discovered are potential targets for in-depth functional studies.

REFERENCES:

- [1] McAdam LP, O'Hanlan MA, Bleustone R, Pearson CM. Relapsing polychondritis: prospective study of 23 patients and review of the literature. *Medicine (Baltimore)* 1976;55:193–215.
- [2] Trentham DE, Le CH. Relapsing polychondritis. *Ann Intern Med* 1998;129:114–122.
- [3] Lang B, Rothenfusser A, Lanchbury JS, Rauh G, Breedveld FC, Urlacher A, Albert ED, Peter HH, Melchers I. Susceptibility to relapsing polychondritis is associated with HLA-DR4. *Arthritis Rheum* 1993;36(5):660–664.
- [4] Zeuner M, Straub RH, Rauh G, Albert ED, Scholmerich J, Lang B. Relapsing polychondritis: clinical and immunogenetic analysis of 62 patients. *J Rheumatol* 1997;24(1):96–101.

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Adaptive immunity (T cells and B cells) in rheumatic diseases

AB0023

SEX-BASED DIFFERENCES IN ASSOCIATION BETWEEN CIRCULATING T CELL SUBSETS AND DISEASE ACTIVITY IN UNTREATED EARLY RHEUMATOID ARTHRITIS PATIENTS

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Background: Genetic association studies strongly support the role of CD4⁺ T cells in promoting RA pathology. In a cohort of untreated early RA patients, we recently demonstrated that the balance of helper T cell subsets in blood of ueRA patients is skewed towards Th2 cells relative to healthy controls.¹ RA has been shown to be a sexually dimorphic condition with current data suggesting that prevalence, disease course and treatment outcome varies between men and women.

Objectives: It is not known if sex-based disparities in immunological factors contribute to the disease process in rheumatoid arthritis (RA). Hence, we examined whether circulating T cell subset proportions and their association with disease activity differed in male and female patients with untreated early rheumatoid arthritis.

Methods: Proportions of T cell subsets were analysed in peripheral blood from 70 ueRA DMARD and prednisolone naïve patients with untreated early Rheumatoid arthritis (50 females and 20 males) and in 31 healthy age-matched controls. Broad analysis of helper and regulatory CD4⁺ T cell subsets was done using flow cytometry. Disease activity in patients was assessed using DAS28, CDAI, swollen joint counts, tender joint counts, CRP and ESR.

Results: Multivariate factor analyses showed that male and female untreated early rheumatoid arthritis patients display distinct profiles of association between disease activity and circulating T cell subset proportions. In male, but not female, ueRA patients Th2 cells showed a positive association with disease activity and correlated significantly with DAS28-ESR, CDAI and tender joint counts. Likewise, proportions of non-regulatory CTLA-4⁺ T cells associated positively with disease activity in male patients only, and correlated with DAS28-ESR. In contrast, there was a negative relation between Th1Th17 subset proportions and disease activity in males only. Proportions of Th1 and Th17 cells showed no relation to disease activity in either males or females. There were no significant differences in proportions of T cell subsets between the sexes in patients with untreated early rheumatoid arthritis.

Conclusions: In conclusion, our findings show sex-based differences in the association between T cell subsets and disease activity in ueRA patients, and that Th2 helper T cells may have a stronger role in the regulation of disease activity in male patients.

REFERENCE:

- [1] Pandya JM, et al. Circulating T helper and T regulatory subsets in untreated early rheumatoid arthritis and healthy control subjects. *J Leukoc Biol* 2016;100(4):823–833.

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AB0024

IGA-EXPRESSING BM PC ARE RESPONSIBLE FOR ENHANCED PHOSPHORYLATION OF BCR-ASSOCIATED KINASES AFTER BCR STIMULATION INDEPENDENTLY OF THEIR CD19 EXPRESSION

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Background: Plasma cells (PC) are considered key drivers of antibody mediated autoimmune diseases. Long-lived PC survive for years in their niches, preferentially in the bone marrow (BM) but also in inflamed tissues.^{1,2} A subset of PC lacking expression of CD19 has been identified.³ Better understanding of factors and pathways involved in survival and maintenance of long-lived PC is needed to find strategies to target PC in autoimmunity since targeting B cells or proliferating cells does not affect already existing PC.⁴ B cell receptor (BCR) signalling is a critical mediator of B cell survival and it was shown before that IgA⁺ and IgM⁺ PC in the BM express a functional BCR suggesting a potential role for the BCR signalling pathway.⁵

Objectives: Expression of BCR associated molecules in BM PC and the response of CD19⁺ and CD19⁻ BM PC to BCR stimulation by anti-IgM/IgA/IgG was assessed to test whether these PC subsets are capable of responding to BCR mediated signals. We further investigated if the PC isotype has an impact on BCR signalling.

Methods: BM samples from patients undergoing routine total hip arthroplasty without systemic immune manifestations were stained for baseline expression of spleen tyrosine kinase (Syk) and Bruton's tyrosine kinase (Btk) as well as for the phosphosites pSyk (Y³⁵²) and pBtk (Y²²³). BM mononuclear cells have been isolated, stimulated with anti-IgM/IgA/IgG and the increase of fluorescence intensity of pSyk (Y³⁵²) and pBtk (Y²²³) was measured by intracellular flow-cytometric analyses. In some experiments, cells have been stimulated with anti-IgA alone and stained for the isotype additionally to the pPTK staining.

Results: Whole BM stainings revealed that both CD19⁺ and CD19⁻ PC express the PTKs Syk and Btk at baseline. Both PC subsets showed the ability to respond to BCR stimulation with enhanced phosphorylation of the PTKs with a clear trend to reduced responsiveness among CD19⁻ PC. Ig staining revealed that IgA expression was identified on both membrane and intracellularly, whereas IgG was

only expressed intracellularly. Co-staining of IgA with pPTKs showed that IgA⁺ PC in both subsets are responsible for enhanced PTK phosphorylation independently of CD19 expression.

Conclusions: CD19⁺ and CD19⁻ BM PC express kinases involved in BCR signaling and respond by enhanced phosphorylation of PTKs upon BCR stimulation with IgA-expressing cells being exclusively responsible for this increase. Further functional consequences of IgA expression in BM PC and autoimmunity remain to be delineated.

REFERENCES:

- [1] Manz RA, et al. Lifetime of plasma cells in the bone marrow. *Nature* 1997;388(6638):133–134.
- [2] Tokoyoda K, et al. Organization and maintenance of immunological memory by stroma niches. *Eur J Immunol* 2009;39(8):2095–2099.
- [3] Mei HE, et al. A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow. *Blood* 2015;125(11):1739–1748.
- [4] Hoyer BF, et al. Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J Exp Med* 2004;199(11):1577–1584.
- [5] Pinto D, et al. A functional BCR in human IgA and IgM plasma cells. *Blood* 2013;121(20):4110–4114.

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AB0025 MTOR PATHWAY ACTIVATION IN LARGE VESSEL VASCULITIS

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Background: Mammalian target of rapamycin complex 1 (mTORC 1) drives the proinflammatory expansion of T helper (TH) type 1, TH17 cells and controls fibroblast proliferation, typical features of large vessel vasculitis (LVV) pathogenesis. Molecular pathways involved in arterial lesions of LVV are unknown.

Objectives: To analyse mTOR pathway activation in LVV (giant cell arteritis and Takayasu arteritis).

Methods: We evaluate pathway activation in the mTORC and the nature of cell proliferation in blood and vessels of patients with LVV compared non-inflammatory aorta by using double immunostaining, western blot and flow cytometry. Finally, using flow cytometry, we study the effect of rapamycin on T cells homeostasis in LVV compared to HD.

Results: Proliferation of both endothelial cells and vascular smooth-muscle cells was shown in vascular lesions in LVV. The vascular endothelium of proliferating aorta vessels from patients with LVV showed indications of activation of the mTORC1 pathway on endothelial cells (S6RP phosphorylation) compared to non-inflammatory aorta (45%^{24,48} versus 10.4% [9.7;14.9] positive S6RP endothelial cells, $p=0.03$). In cultured vascular endothelial cells, sera from patients with LVV stimulated mTORC1 through the phosphorylation of S6RP. Activation of mTORC1 was also found in Th1 and Th17 cells both systemically and in the blood vessels. Patients with LVV exhibited a diminished S6RP phosphorylation in Tregs. Inhibition of mTORC1 pathway with rapamycin, increase Tregs and decrease effector CD4⁺IFN γ ⁺, CD4⁺IL17⁺ and CD4⁺IL21⁺ T cells in patients with LVV.

Conclusions: Our results suggest that the mTORC1 pathway is involved in the vascular lesions of LVV. Targeting mTORC pathway may represent a new therapeutic option in patients with LVV.

Disclosure of Interest: None declared

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AB0026 TLR9 STIMULATION OF ANERGIC HCV-ASSOCIATED ATYPICAL MEMORY B CELLS TRIGGERS RHEUMATOID FACTOR AUTOIMMUNITY BY THE TNF- α PATHWAY

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Background: Hepatitis C virus (HCV) infection contributes to the development of autoimmune disorders, but the mechanisms responsible for HCV-associated autoimmunity are not well understood.

Results: Here we show that TLR9 stimulation of atypical memory (AtM) B cells from patients with HCV-associated cryoglobulinemia vasculitis induce the secretion of IFN γ , TNF α but not IL17A by CD4⁺CD25⁺ effector T cells and stimulate their proliferation. Conversely, they reduce the proliferative capacity of CD4⁺CD25^{hi}CD127^{low}FoxP3⁺ regulatory T cells. TLR9-stimulated AtM secrete TNF α and IgMs with rheumatoid factor activity. We identify a transcriptional

signature specific of TLR9-stimulated AtM, centred on TNF α overexpression. AtM B-cell expansions display intraclonal diversity of mutated IgM with features of antigen-driven maturation. AtM-derived antibodies possess rheumatoid factor activity, with each antibody clone targeting a unique epitope on the human IgG Fc region. AtM antibodies are neither polyreactive nor reactive to ubiquitous autoantigens and importantly, not cross-reactive against HCV antigens including NS3 and E2 proteins.

Conclusions: These data strongly suggest a central role for AtM in defective tolerance of HCV-CV patients through TLR9 reactivation of anergic AtM and production of IgM antibodies with rheumatoid factor activity.

Disclosure of Interest: None declared

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AB0027 SCREENING FOR ANTIBODY REACTIVITY IN EARLY AXIAL SPONDYLOARTHRITIS IDENTIFIES NOVEL ANTIGENIC TARGETS

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Background: Diagnosis of axial spondyloarthritis (axSpA) is challenging since clinical manifestations, such as inflammatory back pain, peripheral arthritis, enthesitis and inflammatory bowel disease, often overlap with other disorders. Despite the use of the genetic marker Human Leukocyte Antigen (HLA)-B27 in axSpA patients, an appropriate serological test is still lacking. Although antibodies are not considered to be a hallmark of axSpA, emerging evidence suggests plasma cells and antibodies to be involved in the disease course¹.

Objectives: Our aim is to screen for antibodies reactive against antigenic targets in plasma of early axSpA patients which may potentially result in novel antibody biomarkers to improve axSpA diagnosis and can enhance the assessment of disease activity, prognosis and therapy response.

Methods: We applied Serological Antigen Selection (SAS), an unbiased and high-throughput antibody profiling procedure based on cDNA phage display. First, a cDNA phage display library was constructed from synovial hip tissue from 3 axSpA patients and screened for antibody reactivity in pooled plasma of early axSpA patients ($n=10$). By performing SAS, we identified antibodies in the axSpA plasma pool that were reactive against 104 different antigenic targets. These targets correspond to both known proteins and novel linear peptides. In a first validation, antibody reactivity against each of these 104 SAS-identified targets was determined in pooled plasma of additional early axSpA patients ($n=50$) and healthy controls (HC, $n=30$). Antigenic targets that showed highest reactivity in axSpA plasma pools were further validated in individual plasma samples of early axSpA patients ($n=71$) and HC ($n=73$) using phage enzyme-linked immunosorbent assay (ELISA).

Results: Increased antibody reactivity against 7 targets was found in pooled plasma of additional early axSpA patients. Further validation of these 7 antigenic targets in individual plasma samples revealed antibody reactivity in 39% of the early axSpA patients (28/71) compared with 21% of the HC (15/73). By forming a biomarker panel with 4 of these targets, specificity could be improved to 88% (9/73 HC) with only a slightly decrease in sensitivity (34%, 24/71).

Conclusions: We identified autoantibody reactivity to novel antigenic targets in early AS patients. In order to establish the true biomarker potential, antibody reactivity against our identified novel antigenic targets will be further validated in an independent cohort of axSpA patients, rheumatic controls and low back pain controls. Identification of antibody reactivity against novel antibody targets in early axSpA patients can contribute to novel biomarkers for an enhanced diagnosis and might provide more insight into the underlying disease pathology, resulting in novel treatment strategies and eventually improve disease outcome in axSpA patients.

REFERENCE:

- [1] Quaden D, et al. *Autoimmun Rev*. 2016 Aug;15(8):820–32.

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