clonal expansion in muscle tissue is significantly correlated with increased CK levels (p=0.03), and tends to correlate with decreased muscle strength (p=0.08).

**Conclusion**: Network analysis of clones in muscle of IIM patients shows shared clusters of sequences across patients. Muscle-restricted TRCβI clones show specific structural features in their T cell receptor, and clonal TCR expansion in muscle tissue is associated with disease activity. These findings indicate that specific clonal T cell responses in muscle tissue act as key players in the pathogenesis of IIM.


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**POS0006**

**AUTOACTIVE B CELLS IN RHEUMATOID ARTHRITIS ARE RECENTLY ACTIVATED AND SHOW LARGE EXPANSIONS OF CXCR3+ ANTIBODY-SECRETING CELLS**

**Keywords**: Adaptive immunity, Cell biology, Rheumatoid arthritis

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**Background**: Many autoimmune diseases (AIDs) are characterized by aberrant, autoreactive B cell responses and autoantibody production. Rheumatoid arthritis (RA) is a common AID in which autoantibodies recognize post-translational modifications (PTMs), collectively called anti-modified protein antibodies (AMA), are intimately associated with disease pathogenesis. Previously, we observed that memory B cells (mBC) against one of these PTM-antigens, citrulline, are highly activated. This phenotype persists for years in patients with established disease [1]. The evolution, dynamics and more detailed phenotypic characteristics of the PTM-directed B cell compartment are still ill defined, however, partly owing to challenges linked to the visualization of such cells in human samples, and the limitation of conventional flow cytometry. Here, we visualized the autoactive B cell response against three different PTM antigens (citrulline, homocitrulline, acetyllysine) by spectral flow cytometry, allowing us to address cross-reactivity on the B cell level and to perform deep phenotypic profiling of individual B cell compartments.

**Objectives**: To develop a detailed understanding of the autoactive B cell response against PTM-antigens in RA with the aim to elucidate features associated with its cross-reactivity, state of activation in the disease context, and its remarkable persistence for years without signs of exhaustion or decay.

**Methods**: We developed a spectral flow cytometry staining approach using differentially labelled streptavidin molecules coupled to individual PTM-antigens. B cells reactive to each antigen were identified together in individual samples by double staining for each antigen, while at the same time excluding cells reactive to either arginine or lysine control peptides. This combinatorial staining was further extended by the concomitant visualization of tetanus-toxoid specific B cells and expression levels of various activation and homing markers, and applied to peripheral blood samples of patients with established RA. Importantly, we performed intracellular stainings, allowing us to additionally enumerate and characterize circulating plasmablasts and plasmacells.

**Results**: We successfully visualized autoactive B cells directed against different PTM-antigens and their subset distribution in individual patient samples. We observed extensive cross-reactivity against all three PTM antigens with citrulline as dominant antigen. Unsupervised clustering revealed several memory B cell populations, with most autoreactive B cells populating the most recently rulline as dominant antigen. Unsupervised clustering revealed several memory B cell compartments.

**Conclusion**: Our results identify citrulline as the most prominent antigen recognized by AMAA-expressing B cells. The study highlights the recent and prolonged activation state of PTM-reactive mBC and their continuous differentiation towards ASC. Unspecific ASC may home towards CXCR3 ligands known to be abundant in the synovial compartment. This degree of mBC activation was also found in patients with low clinical disease activity scores, indicating that conventional therapeutic interventions may suppress inflammation but fail to silence the most disease-specific autoactive B cell response in RA. Targeting this compartment may therefore be relevant for future therapeutic interventions aiming at the induction of tolerance and/or permanent cure. Finally, the combinatorial staining approach presented will be a valuable tool to delineate the development of PTM-directed autoimmunity in the pre-disease phase as well as its state of activation in phases of sustained drug-free remission and/or during beallergicogenic interventions.


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**POS0007**

**KYY-101, A FULLY HUMAN CD19 CAR T CELL GENERATED FROM AUTOIMMUNE PATIENT LYMPHOCYTES, DEMONSTRATES CAR-MEDIATED AND CD19-DEPENDENT ACTIVITY AGAINST AUTOLOGOUS B CELLS**

**Keywords**: Adaptive immunity, Systemic lupus erythematosus, Autoantibodies

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**Background**: A significant unmet medical need remains in the treatment of relapsed and/or refractory B cell-driven autoimmune diseases, including lupus nephritis (LN). The presence of autoantibodies is a hallmark of such diseases and implicates dysregulated B cell function in their pathogenesis. The central role of B cells in these diseases is also supported by the presence of B cells in diseased tissues and the efficacious responses reported with biologic therapies that target B cells. KYY-101 is an autologous CD19 CAR-T cell therapy that depletes pathogenic B cells. Importantly, the CD19 CAR utilized in KYY-101 was previously tested in B-cell lymphoma patients and demonstrated efficacy with an improved safety profile [1]. Since CD19 CAR-T cells target and lyse B cells in both circulation and tissues, a more complete depletion of autoactive B cells is expected with KYY-101, resulting in better disease control and clinical remission than the current immunotherapies.

**Objectives**: To demonstrate the CAR-mediated and CD19-dependent activity of KYY-101 against autologous, patient-derived primary B cells.

**Methods**: Autologous CD4+ and CD8+ T cells were enriched from healthy donors (HD), systemic lupus erythematosus (SLE), LN or other autoimmune patients. KYY-101 CAR T cells were transduced with a lentiviral vector encoding a fully human single-chain variable fragment (scFv) CD19-targeting domain, a CD8 hinge and transmembrane domain, a CD28 cytoplasmic costimulatory domain, and a CD3ζ cytoplasmic domain. The CAR-mediated and CD19-dependent activity of KYY-101 was monitored in vitro in a set of cytotoxicity, cytokine release and proliferation studies, in response to either CD19+ target cell lines or autologous, patient-derived primary CD19+ B cells.

**Results**: Following a 24-hour incubation, KYY-101 generated from HDs or autoimmune patients induced greater and dose-dependent cytotoxicity of both the human CD19+ NALM6 cell line and autologous, patient-derived primary B cells than their respective untransduced control T cells. Moreover, an effector cell dose-dependent increase in the production of cytokines such as IFNγ was also observed following co-culture. In contrast, no differences in cytotoxicity nor cytokine production were observed when CD19- target cells (K562 or U937 cells) were co-cultured with KYY-101 or untransduced control T cells. In addition, following a 96-hour incubation, KYY-101 generated from HDs or autoimmune patients proliferated when co-cultured with the NALM6 cells and autologous, patient derived primary B cells, whereas substantially lower levels of proliferation were observed in the untransduced control T cells co-cultured with NALM6 or autologous, patient-derived primary B cells in or KYY-101 and untransduced control T cells co-cultured with the CD19+ cell lines K562 and U937.

**Conclusion**: KYY-101 generated from autoimmune disease patient lymphocytes demonstrates CAR-mediated and CD19-dependent activity against autologous, patient-derived primary B cells and thus represents a novel therapeutic option for the depletion of pathogenic B cells in autoimmune patients.

STRATIFICATION OF NEGATIVE, SEROCONVERTED AND PERSISTENTLY POSITIVE THROMBOTIC PRIMARY ANTIPHOSPHOLIPID SYNDROME PATIENTS WITH DISTINCT FOLLICULAR HELPER T CELLS SUBSETS

Keywords: Anti-phospholipid syndrome, Autoantibodies, Adaptive immunity

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Background: Primary antiphospholipid syndrome (PAPS) is caused by autoantibodies targeting protein-binding phospholipids (aPL) [1]. Follicular helper T (Tfh) and follicular regulatory T cells (Tfr) are critical for antibody production in germinal centres [2], but their role in PAPS is scarcely studied. Some patients remain persistently positive for aPL, while others become seronegative during disease progression [3]. Whether aPL status mirrors immunological changes over time is not known.

Objectives: To phenotypically characterize Tfh/Tfr subsets in PAPS and explore the association with previous/current aPL status.

Methods: We prospectively recruited 73 adult thrombotic PAPS patients (Sidney criteria; ≥1 year disease duration) and 24 age- and sex-matched healthy donors (HD). Regardless of lupus anticoagulant (LA) status, patients were categorized in three groups according to aPL (anticardiolipin [ACL] IgM and/or IgG; β2-glycoprotein [β2GPI] IgM and/or IgG) profile: NN – previous and current aPL negative, ie, only positive for LA (n=40); PP – previous and current aPL positive (n=20); and PN – previous aPL positive, currently aPL negative (n=13). We considered aPL <30 U/ml as negative (ELISA immunoassays). Tfh (CD4+FoxP3+CD25+CD45RO+CXCR5+), activated Tfh (PD1+ICOS+ Tfh), Tfh-like (CXCR3+CXB+ Tfh), Tfh2-like (CXCR3-CCXα6 Tfh), Treg (CD4+CD20+FoxP3+), and Tfr (CD4+CD25+FoxP3+CXB+) cells were analysed by flow cytometry using cryopreserved peripheral blood mononuclear cells. GraphPad Prism version 8 software was used for statistical analysis and p values <0.05 were considered significant.

Results: Most patients were women (n=43, 59%), with a mean age at disease onset of 42±13 years, and a mean disease duration of 8±7 years. Venous thrombosis was the most common first manifestation (53%), and pregnancy morbidity was present 10% of women. At presentation, aPL positivity was as follows: LA, 70%; ACL IgG and/or IgM, 44%; β2GPI IgG and/or IgM, 33%; triple positive, 16%. Circulating Tfh and Treg cells were comparable between HD and all APS patients (Figure 1A and 1B). Among APS, PP patients had higher frequency of Tfh compared to NN (p=0.047) but not with PN patients. Within the Tfh subset, activated Tfh cells were significantly increased in PP compared to NN (p=0.005) and PN patients (p=0.004), whereas no difference was seen between the latter group and NN patients, and between HD and all APS patients (Figure 1C). The frequencies of Tfh1-like and Tfh17-like cells were comparable between all groups, but Thf2-like cells were decreased in PP compared to NN patients (p=0.008), and similarly distributed among all other groups (Figure 1D to F). In the regulatory compartment, circulating Tfr and Tfr/Tfh ratio were significantly increased in PP patients compared to NN (p<0.001; p=0.001) and PN patients (p=0.004; p=0.046) (Figure 1G and 1H). No difference was observed between NN and PN patients nor between HD and all APS patients.

Conclusion: Our preliminary results show that in thrombotic PAPS, imbalance of circulating Tfh and Tfr subsets change according to aPL profile. Increased PD1+ICOS+ Tfh and Tfr cells, and Tfh/Tfr ratio indicates ongoing humoral response in persistently positive patients. These changes are lost after seroconversion with T follicular cells subsets being similar to persistently negative PAPS patients.

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